Investigation of biochar stability by means of gas isotopic measurements

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List of abbreviations / Abkürzungverzeichnis

C	carbon
^{12}C ,^{13}C	carbon isotopes (stable)
CO_2	carbon dioxide
FM	fresh matter
DM	dry matter
H	hydrogen
O	oxygen
oDM	organic dry matter
Pyro	pyrolysis char (untreated)
HTC	hydrothermal carbonisation char (untreated)
Pyro-ferm	pyrolysis char post-processed by fermentation
HTC-ferm	HTC char post-processed by fermentation
Gluc	glucose
Actino	Actinobacteria
Acido	Acidobacteria
Alpha	Alphaproteobacteria
Beta	Betaproteobacteria
Gamma	Gammaproteobacteria
Firmi	Firmicutes
ppm	part per million
Pg	petagrams = 10^{15} g
yr	years
yr^{-1}	$years^{-1}$

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1. Introduction

Carbonisation of organic matter and its subsequent application to soil in the form of char has been proposed for the double aim of long-term carbon storage and of soil amendment (Lehmann and Joseph, 2009; UBA, 2016). Several examples of long-lived charcoal deposits have been reported (Preston and Schmidt, 2006; N. Singh et al., 2012), as well as positive effects of soil carbon enrichment on plant growth (Major et al., 2010). These findings seem to support proposals to convert different types of biomass, especially agricultural residues, to products which can be used by farmers to increase their crop yield. However, these findings cannot always be generalised, because the reported effects are strongly dependent on the kind of feedstock, which was carbonised, on the production process parameters, on the soil type, on the kind of crop and on weather conditions (Jeffery et al., 2011). Moreover, it is not fully understood how the carbonisation process is related to the structure and functionality of the chars, as well as how the chars interact biochemically with the soil ecosystem where they are inserted. On the one hand, chars can affect the abundance and activity of soil microorganisms and thus encourage or inhibit the metabolisation of soil organic matter at the field scale. On the other hand, microorganisms can interact with the surface of chars and contribute to their degradation, thus playing a role in defining their reactivity and their stability. A careful inspection of such interactions is an essential part when evaluating the suitability of chars for an application on a field.

1.1 Research questions

The object of the present work is to investigate the interactions of selected chars with a pre-existing soil ecosystem, with particular focus on the decay of the chars and their effect on the microbial activity and soil carbon degradation. The aim is to answer the following key questions:

- Which factors affect the degradability of chars?

Within this study, the following factors were tested: char production process; char post-processing; char ageing; addition of nutrients; addition of labile carbon.

- How do chars affect a soil ecosystem?

Within this study, the following parameters of the soil ecosystem were inspected: soil total respiration; soil respiration dynamics; soil carbon abundance; microbial abundance; microbial population dynamics.

1.2 Structure of the present work

This thesis consists of three experimental works which have been published as well as proposed for publication in international peer-reviewed journals.

Article 1 (Lanza et al., 2015) describes a laboratory incubation investigating the soil respiration dynamics of soil amended with different native and post-processed chars and the effect of mineral nitrogen fertiliser as additional nutrient source. It was published in 2015 in the journal *Pedosphere* (vol. **25**(5), pp. 761-769).

Article 2 (Lanza et al., 2016) describes a laboratory incubation investigating the soil respiration and the microbial population dynamics of soil amended with the same chars and the effect of glucose as additional energy source. It was published in 2016 in *Soil and Tillage Research* (vol. **164**, pp. 18-24).

Article 3 (Lanza et al., submitted) describes a field investigation investigating the soil carbon content, respiration and isotopic composition of soil amended with the same chars. It has been submitted to the journal *Biology and Fertility of Soils*.

2. State of the art

2.1 Climatic change and greenhouse effect

The climate is evolving towards higher temperatures worldwide (GISTEMP Team, 2016; Hansen et al., 2010). That is a consequence of the greenhouse effect, that is, the retention of the warmth released by the Earth's surface due to the presence in the atmosphere of infrared-absorbing gases such as water vapour (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), chlorofluorocarbides (CFCs) and other fluorinated compounds (Forster et al., 2007).

In particular, the concentration of CO_2 in the atmosphere has increased up to the actual 400 ppm in 2016 (Dlugokencky and Tans, 2016), a value considerably higher than the 280 ppm before the Industrial Revolution (Keeling et al., 2001) or the 200-280 ppm level which was maintained in the last 420000 years of the prehistory (Petit et al., 1999) and is still increasing with a rate of 2 ppm yr^{-1} corresponding to 2 Pg CO_2-C yr^{-1} (Ciais and Sabine, 2013). The biggest source for atmospheric CO_2 is the extraction and burning of fossil fuels for heating, transportation or industrial processes. The contribution of agriculture is also considerable (Smith et al., 2008), deriving mostly from land use change and degradation of agricultural residues (litter, straw, wood, digestate) left on a field after harvest or applied subsequently as soil amendment contributes.

Atmospheric CO_2 is mostly captured by ocean water (Sabine et al., 2004; Siegenthaler and Sarmiento, 1993) and converted to carbonic acid (H_2CO_3), leading to an acidification of water (IPCC, 2005). It can also be captured by autotrophic organisms (eukaryotic

plants and procaryotic algae), to be transformed into glucose ($C_6H_{12}O_6$) by photosynthesis. At the present stand, these natural ways of carbon capture are not sufficient to compensate the enormous anthropogenic carbon release (Ballantyne et al., 2012). Therefore solutions have to be found to reduce the atmospheric concentrations of carbon dioxide at a safe level, which has been identified to be under 350 ppm (Hansen et al., 2008). One necessary step is a change in our lifestyle, involving a drastic reduction of the consumption of fossil fuels and raw materials. The impact of agriculture can also be reduced by a more efficient cycling of resources. In particular, biomass residues need to be converted to a more stable form before application onto a field, in order to reduce its further decomposition and maintain the included carbon and nitrogen in the soil, thus improving soil quality.

2.2 Background information about chars

One way to transform organic carbon into a more stable form is carbonisation, or charring. The main products of carbonisation, generally known as chars, consist mostly of carbon arranged in a highly stable polyaromatic structure (Knicker et al., 2008; McBeath and Smernik, 2009; Titirici et al., 2008), which can last for even millennia. Due to their porous structure and the presence of functional groups on the surface (Budai et al., 2014), chars have the capacity to retain water and nutrients (Gul and Whalen, 2016) and release them over time.

Several deposits of ancient pyrogenic carbon have been found all over the world and are well-known for their fertility and stability. The most famous are the *Terra preta do indio* (Glaser et al., 2001), a “black earth” containing rests of charcoal and human and animal

manure, which has been found within the Amazonian basin on the sites of ancient settlements of the indigeneous population, and the *Sambaqui* (Gaspar et al., 2008; Teixeira et al., 2012), a soil formation containing charcoal, bones and sea-shells, found mostly on funerary sites at the Brasilian coast. Terra preta imitates, comprising charcoal and nutrient-rich substrates such as compost, are nowadays available on market or produced by garden plot holders to be used in horticulture (Downie et al., 2011; Factura et al., 2010).

2.2.1 Production and potential applications

Different techniques have been developed to carbonise organic matter, such as pyrolysis, hydrothermal carbonisation (Hu et al., 2008; Libra et al., 2011; Titirici, 2013), vapothermal carbonisation (Funke et al., 2013), etc. In principle chars can be made out of any type of organic matter, though pyrolysis requires previous drying of the feedstock. Production of chars from agricultural residuals such as straw, manure, wood chips is particularly promising. In the case of animal residues such as manure, charring has the additional advantage of sterilising it, thus reducing the risk of biological contamination.

The obtained chars may be utilised in several ways. Their first historical application was as a fuel for cooking, heating, or moving engines. The byproducts of carbonisation, such as syngas and pyrolysis oil, can also be used as fuels. Other potential applications of chars arise from their physical properties. Their sorption properties, due to their porous structure, can make them suitable to produce filter materials to remove contaminants from air, wastewater, or polluted soils. Finally, their capability to bind water and

nutrients has suggested their employment as a soil amendment (UBA, 2016). Chars can be therefore used for mulching, to substitute peat in growing media, or to be integrated within agricultural soils.

1.1.1 Chars or biochars?

A large international community is active in the testing and regulation of chars suitable for soil application, which are commonly named "biochars". An official regulation issue by the European Biochar Foundation (EBC, 2016) defines "biochar" as

> *"[…] a heterogeneous substance rich in aromatic carbon and minerals. It is produced by pyrolysis of sustainably obtained biomass under controlled conditions with clean technology and is used for any purpose that does not involve its rapid mineralisation to CO_2 and may eventually become a soil amendment."*

and sets the following conditions: carbon content > 50 %, molar H/C ratio < 0.7, molar O/C ratio < 0.4. HTC chars often do not fulfil these requirements and therefore are not considered as biochars by some authors (Schimmelpfennig and Glaser, 2012). For that reason, to avoid any misunderstandings, the more generic term "char" is used throughout this work to designate the carbonised substrates, coherently with Libra et al. (2011).

2.3 Interactions of chars with soil ecosystems

The present work focuses on the usage of chars for soil amendment and long-term carbon storage. In order to evaluate the suitability of a specific char for these purposes,

it is highly important to forecast the duration of the expected effects, which is obviously related to the longevity of the char itself.

2.3.1 Stability and degradability of chars

Soil carbon can be degraded through mechanical processes generally known as "weathering", like thermal stress and erosion by wind or water; or through chemical processes such as acidification. Biotic processes are also very important for carbon degradation and the most important role is played by microorganisms (Bacteria, Archaea and Fungi), which possess enzymes capable to digest several forms of carbon, convert it subsequently into new biomass and release it as CO_2 under aerobic conditions or CH_4 under anaerobic conditions. Different phyla of microorganisms have been shown to prefer different substrates. The microbial activity depends on environmental conditions such as temperature (Lloyd and Taylor, 1994; Nguyen et al., 2010) or pH and also on the abundance of energy sources like sugars or additional nutrients like nitrate, ammonium and phosphate. As a result, carbon is released into the atmosphere, mostly as CO_2, or leached into the groundwater as dissolved organic or inorganic carbon.

2.3.2 Estimates of char age or longevity

The longevity of a char can be estimated with field experiments or laboratory incubations by measuring the total carbon remaining at certain time points after application, or by quantifying the carbon losses (Lanza and Kern, 2016). Within a controlled system with constant environmental parameters, the time decay of the carbon can usually be described by a 1st order kinetics, which yields a very simple mathematical formula for single exponential decay (Eqn. 1) or double exponential decay

(Eqn. 2), which assumes the soil/substrate carbon to be composed of one or two carbon pools (Cheng et al., 2008; Liang et al., 2008):

(1) $$C_{\text{lost}}(t) = C_0 \cdot \left(1 - \mathrm{e}^{-k \cdot t}\right), \text{ or}$$

(2) $$C_{\text{lost}}(t) = C_1 \cdot \left(1 - \mathrm{e}^{-k_1 \cdot t}\right) + C_2 \cdot \left(1 - \mathrm{e}^{-k_2 \cdot t}\right)$$

Such models allow quantifying the degradability of carbon with a simple quantity such as the decay kinetic coefficient k (yr^{-1}), related to the microbial activity, the mean residence time $\tau = 1/k$ (yr) or the half-life $t_{½} = (\ln 2)/k$ (yr). For a heterogeneous sample, the coefficient related to the recalcitrant pool (the smaller k) is usually the most interesting. The sum of the amplitudes C_i needs not be restricted to be equal to the initial amount of carbon, allowing for a fraction of "immobile carbon".

If the time dependence of the lost carbon $C_{\text{lost}}(t)$ is more complex, a precise mathematical reconstruction of the kinetics is often not possible. In such case an approximate estimate of the decay coefficient can be obtained by normalising the decay rate by the initial amount of carbon:

(3) $$\widetilde{k} = \frac{1}{C_0} \cdot \frac{\Delta C}{\Delta t}$$

$\widetilde{k}$ is a valid approximation for k, as long as the relative carbon loss is small. A variation of k over time is an indicator of a dynamic evolution of the microbial community over time.

2.3.3 Effects of chars onto soil systems

Application of chars onto soil, besides the desired increase of soil carbon, water holding capacity and cation exchange capacity, can have several side effects on the soil structure, the abundance and vitality of soil fauna and micro fauna, the root development of plants and their resistance to diseases. All these phenomena need to be investigated carefully before bringing a new product onto the market.

In particular, char can have an effect on the composition and activity of the soil microbial community. As a consequence, chars may also influence the decay of carbon contained in the soil or in some other substrates. This phenomenon is called “priming” (Kuzyakov, 2010) and can be quantified via the difference between the loss of soil carbon with and without chars. Therefore priming is said to be positive in case of an increase of the metabolisation or mineralisation of another substrate, or negative in case of inhibition. Priming of chars has been reported in both directions; it is more often negative, especially for chars produced from crop residues, on most soil types, but it is normally positive on sandy soils (Wang et al., 2016).

2.3.4 Issues related to estimates of char age or longevity

All these studies rely on the extrapolations from few days/months/years of observation, which try to represent phenomena occurring ideally on the scale of decades and centuries. As the decay dynamics are initially dominated by the presence of the easily degradable compounds, the apparent decay rate decreases with increasing length of the incubation study. Therefore most investigations about char stability (Bai et al., 2013; Qayyum et al., 2012; Schulze et al., 2016; Zimmerman, 2010) underestimate the half-life.

Another difficulty is to distinguish between the carbon coming from the chars and the carbon deriving from the soil itself, which can be different than the respiration from the control soil if priming occurs. That issue can be somehow overcome by some independent measurement of some quantity related to the composition of the mixture soil-C/substrate-C. Promising results have been obtained with the detection of the abundance of the heavier isotopes of carbon, ^{13}C and ^{14}C, in presence of correspondingly enriched substrates.

An additional difficulty arises for field investigations, which as opposed to laboratory incubations suffer from the variability of climatic factors, soil inhomogeneity and irregular presence and activity of soil biota.

3. Experimental approaches

3.1 Framework and "scale" of the experiments

The thesis work has been carried out within the cooperative project "Biochar in agriculture: Perspectives for Germany and Malaysia" financed by the Leibniz Association, aiming to study the suitability of chars as a soil amendment from the ecological, economical and social point of view. Seven working groups from six research institutions located in Germany and one in Malaysia were involved and performed different investigations with the same material.

This present work is based onto two years of measurements on a dedicated field experiment and three incubation studies using the same soil and the same substrates which have been used in the field experiment. The research field was located in Berge (Kreis Havelland, Brandenburg, Germany, 52° 63′N, 12° 80′E) and was directed by the Institute of Agricultural and Urban Ecological Projects (IASP). The incubation studies were performed at the facilities of the Leibniz Centre for Agricultural Landscape Research (ZALF) at the Institute of Landscape Biogeochemistry. The soil used for the experimental work was classified as a Cambisol with a sandy loamy texture. All chars were made from the same feedstock – maize silage; a part of them underwent a fermentation post-processing in order to improve their biocompatibility and increase their surface area (Mumme et al., 2014; Sänger et al., 2016).

3.2 Soil respiration as a measure for carbon decay

The most important product of carbon degradation is carbon dioxide, CO_2, released into the atmosphere. It can be easily measured in relationship to a specific air and soil volume, contained in a chamber, which can be dynamic (with air feed from outside) or static, transparent (to allow for photosynthesis) or opaque. Our field investigation was conducted through a set of closed opaque chambers, while our laboratory incubations were performed in a dynamic system kept under constant temperature, humidity and air flux.

The abundance of emitted CO_2 is usually expressed as molar fraction X_{em} (in ppm) and can be measured by a gas spectrometer (usually IR), a gas chromatograph or through precipitation of a NaOH solution followed by titration with HCl. From X_{em}, the instantaneous flux Φ can be calculated as

(4) $$\Phi = \frac{1}{\Omega} \cdot X_{em} \cdot W \cdot \frac{MM}{VM}$$ for a dynamic chamber with air flux $W = \frac{dV}{dt}$;

(5) $$\Phi = \frac{1}{\Omega} \cdot \frac{dX_{em}}{dt} \cdot V \cdot \frac{MM}{VM}$$ for a static chamber of volume V;

where MM = 12.0107 g mol^{-1} is the molar mass of carbon; VM is the molar volume of an ideal gas at given temperature T (VM = 22.414 L mol^{-1} at T = 273.15 K, VM = 24.055 L mol^{-1} at T = 293.15 K) and Ω is a quantity used for normalisation, which can be the area $Æ$, the soil mass m or the quantity of soil carbon C included into the chamber.

If the flux variations between sampling points are smooth enough, a cumulated flux can be calculated as

(6) $$\Phi_{\mathrm{cum}}(t) = \sum_{t'<t} \Phi(t') \cdot \Delta t'$$

which corresponds also to the cumulated mineralised carbon $C_{\mathrm{lost}}(t)$, if all other decay processes can be neglected.

3.3 Stable isotopes for source discrimination

Carbon possesses two stable isotopes, ^{12}C and ^{13}C, whose natural abundances are in a ratio *R* around 10^{-2}. Variations of this value, usually of the order of few parts per thousand (‰), can be used to identify different carbon pools having a different ^{13}C abundance. In particular, all material deriving from C_4 plants are ^{13}C-enriched compared to material from C_3 plants. This can be explained by different isotopic fractionation in the C_3 and C_4 photosynthesis (Farquhar et al., 1989; Glaser, 2005; O'Leary, 1988). For this investigation, chars from maize (C_4 plant) and a soil mainly of C_3 origin were used as isotopically distinct carbon sources.

Stable isotopes can be measured via mass spectrometry or with newly developed optical techniques, such as cavity ring-down spectrometry or optical feedback cavity enhanced absorption spectroscopy, which exploit the difference in absorption bands of isotopologues and compensate the low concentration of the rare isotope through a multiplication of the optical path.

3.4 qPCR for identification of microbes

The microbes present in a soil ecosystem can be distinguished based on the presence of specific genes (*fingerprints*) on their DNA. The quantification of fingerprint genes

contained in a DNA sample extracted from the soil can therefore give information about the microbial *phyla* playing the major role. A powerful technique which allows quantifying selected DNA strains is real-time quantitative polymerase chain reaction qPCR. (Büks et al., 2016; Diehl et al., 2013; Fierer et al., 2005)

4. Short-term response of soil respiration to addition of chars: Impact of fermentation post-processing and mineral nitrogen

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Abstract

The biodegradability of chars derived from pyrolysis or hydrothermal carbonisation (HTC) was studied in short-term dynamic incubation experiments under controlled conditions. CO_2 emissions from soil-char mixtures in combination with solid digestate or mineral N fertiliser were measured in dynamic chambers for 10 d. Compared to the original material (maize straw), pyrolysis and HTC chars showed significantly lower CO_2 emissions and slower decay dynamics; and compared to the soil control, HTC char increased soil respiration to a significant extent, while pyrolysis char did not. The addition of mineral N resulted in a delayed respiration dynamics for HTC char, while the addition of digestate resulted in an increase in the respired CO_2 for pyrolysis char and a

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decrease for HTC char. For the first time, a peculiar two-stage decay kinetics was observed for HTC char, indicating a highly inhomogeneous substrate consisting at least of two C pools.

Keywords

biodegradability, CO_2 emission, decay dynamics, hydrothermal carbonisation char, pyrolysis char

4.1 Introduction

The application of biochar, carbonised organic matter, to soils has been proposed as a method for the long-term storage of photosynthetically fixed carbon (C) in the environment that, at the same time, will provide agronomic benefits such as the improvement of soil properties (Lehmann et al., 2006; Schulz and Glaser, 2012). Biochar in soil can increase the stability of soil aggregates and the availability of nutrients, which in turn have positive effects on plant growth such as an increase in plant biomass (Biederman and Harpole, 2013; Lehmann et al., 2006) and variable effects on abundance and composition of soil fauna and microfauna, depending on environmental conditions (Atkinson et al., 2010; Lehmann et al., 2011). The extent, or duration of the effects of biochar, however, is strongly dependent on its stability, which can be quantified by the decay half-life ($t_{½}$). Suitable values for $t_{½}$ should be at least on the scale of several decades for a practical utilisation of biochar. Some studies give evidence for such a reasonable half-life (Lehmann et al., 2006; Qayyum et al., 2012; N. Singh et al., 2012),

although they all necessarily rely on an extrapolation from investigations of limited duration of few months or years.

Biochar can be produced by pyrolysis or hydrothermal carbonisation (HTC) (Libra et al., 2011). HTC chars have been shown to be less stable than pyrolysis chars from the same feedstock (Bai et al., 2013; Qayyum et al., 2012) and to contain and initially release toxic substances (Becker et al., 2013; Hale et al., 2012). Therefore, at least for HTC char, a post-processing may be necessary before application on the field. A biological post-processing by means of anaerobic fermentation has been recently introduced (Mumme et al., 2014) to improve its biocompatibility and stability. However, the effect of this post-processing on the stability and decomposition dynamics of chars in soils is not well understood yet and needs more detailed evaluation. Application of biochar to cultivated soils must take into consideration not only direct effects, but also possible interactions with conventional agricultural treatments, such as fertilisation. For example, biochar was found to have significant effects on the nutrient cycles in soil as shown by Clough et al. (2013), who reported reductions in NO_3^- leaching and NH_3 emission.

The decay of organic matter in soil is an extremely complex process and usually investigated indirectly, by tracing one or several fluxes from a limited matter pool over time. For the decay of carbon-rich substrates such as biochar, mineralisation to CO_2 is the prevalent mechanism. Thus, quantifying the CO_2 emissions over time is therefore considered a suitable experimental approach for characterising the degradation dynamics of biochar added to soil (Sagrilo et al., 2015). In most cases, static incubations have been used to study the degradation dynamics of biochar in soil (Bruun et al., 2012; Zavalloni et al., 2011). We, however, investigated the CO_2 release from several soil-char

mixtures in a dynamic system with continuous air exchange as shown by Bai et al. (2013) and Farrell et al. (2015). This approach mimics the climatic conditions in the field more closely and allows higher sampling volumes as well as a more frequent data acquisition. The dynamic approach can, therefore, increase the time resolution, which is crucial for investigations that focus on decay dynamics. Within a few days of investigation, qualitative and semi-quantitative information can already be achieved (Heinemeyer et al., 1989; Kautz et al., 2004). Although the exact time scales of long-term physical phenomena cannot be obtained in this way, short-term studies are helpful to compare different treatments and to gain insight into features of the initial decay dynamics. In the case of slowly-decaying substrates, such as biochar, such studies can provide invaluable support for early decision making on which feedstock, production parameters or post-treatments are more advantageous for later application of biochar to soil.

As main substrates in the present study, we used a char produced by conventional pyrolysis, and a char produced by HTC, both from the same feedstock, *i.e.,* maize silage. Furthermore, maize straw, solid digestate from a mesophilic maize silage-fed biogas reactor, and a soil control without any substrate addition were tested. The aims of this study were to test the following hypotheses:

i) Soil respiration will decrease in the following order: straw > HTC char > pyrolysis char > control.

ii) The heterogeneous composition of chars results in sequential stages of decomposition, which are already detectable in short-term studies.

iii) Fermented chars are less biodegradable compared to chars without post-processing.

iv) Mineral N applied to HTC and pyrolysis chars will increase soil respiration.

4.2 Materials and methods

4.2.1 Chars and fermentation post-processing

A pyrolysis char and a HTC char, both from the same feedstock, *i.e.,* maize silage, were used as the main substrates in the present study. Furthermore, maize straw, solid digestate from a mesophilic maize silage-fed biogas reactor, and a soil control without any substrate addition were also tested. Maize straw samples were taken from an experimental field site located in Braunschweig, Germany (Becker et al., 2014), ground in an ultra-centrifugal mill (0.75-mm sieve) and stored until used. Pyrolysis char (REW, Quakenbrück, Germany) was produced in a continuous reactor (600 °C, 30 min) and quenched by means of water sprinkling. HTC char (AVA-CO_2, Karlsruhe, Germany) was produced in a one-pot batch reactor (210 °C, 23 bar, 8 hours) and separated by means of a chamber filter press. Solid digestate (Biowork, Groß Kreutz (Havel), Germany) was collected from a batch-wise solid-state process mesophilic biogas reactor fed with maize silage and operating at 35 °C. A fermentation post-processing was applied to a part of the pyrolysis and HTC chars, as follows: the char was mixed with the digestate in a mass ratio 2:1 and fermented for 4 weeks under mesophilic conditions (35 °C) at the biogas facilities of the Leibniz Institute for Agricultural Engineering, Potsdam, Germany (Mumme et al., 2014), yielding mixtures of fermented char and digestate, in the

following referred to as “post-processed pyrolysis char” or “post-processed HTC char”. After production, all chars and the digestate were stored at -20 °C. A few weeks before the experiments started, the samples were unfrozen, oven-dried for 48 h at 105 °C, ground up to a fine powder and stored at 4 °C. The physico-chemical properties of the substrates used are reported in Table 4.1. For further interpretation, the H:C and O:C molar ratios were calculated for all substrates of treatments in this study and also for the two char-digestates mixtures before the post-processing.

Table 4.1 – Physico-chemical propertiesof the substrates used in this study.

Substrate	pH	DM (mg g^{-1} FM)	oDM	C	H	N	O	H:C	O:C
			----------(mg g^{-1} DM)----------						
Straw	6.29	939	926	464	69	14	377	1.77	0.61
Pyro	9.72	973	837	756	18	17	44	0.29	0.04
HTC	5.18	984	966	636	54	23	250	1.03	0.29
Digestate	7.77	948	678	398	53	37	185	1.58	0.35
Pyro-ferm	9.19	957	727	573	32	27	92	0.66	0.12
HTC-ferm	6.69	969	720	548	52	29	87	1.14	0.12

4.2.2 Preparation of soil-substrate mixtures

The soil used was taken from the top layer (0–15 cm) of an experimental field located in Berge (Kreis Havelland, Brandenburg, Germany), which represents a typical site of the glacial terrain of north-eastern Germany. It was a loamy sand (Haplic Cambisol) with the following texture: 712 mg g^{-1} sand (> 630 µm), 222 mg g^{-1} silt (2--630 µm) and 66 mg g^{-1} clay (< 2 µm). The soil pH was 4.72, while the contents of C, hydrogen (H), nitrogen (N) and oxygen (O) were 6.26, 1.59, 0.55 and 6.16 mg g^{-1} of the dry soil.

The field-moist soil (dry mass = 95 %) was sieved and stored at 4 °C in a container until analysis. After equilibration (2 d, 20 °C), soil was mixed with either straw meal, char,

fermented char, or digestate (< 2 mm particle size, 5 mg dry matter (DM) g^{-1} soil) by means of a kitchen mixer. Mineral N fertiliser (calcium ammonium nitrate, 27 % N; 1.7 mg DM g^{-1} soil) was added in the treatments, where the fertilisation effect was studied. Due to the high number of variants and replicates, the experiments run in two incubations sequentially. The effect of char fermentation was investigated in a first incubation run and the effect of N addition in a second separate run.

4.2.3 Analysis of soil respiration

Soil-substrate mixtures (100.5 g) were incubated in 3 replicates in Plexiglas tubes (4 cm diameter) for up to 300 h at 20 °C and constant soil moisture in a constant air flow ($\dot{W}$ = 80 mL min^{-1}), while the molar fraction of the emitted CO_2 (X_{em}, in µmol mol^{-1}) was measured continuously (with a periodicity Δt = 2 hours) using an automated dual-channel infrared gas analysis system (Heinemeyer et al., 1989).

4.2.4 Calculation of respiration kinetics

Soil respiration response was quantified as the cumulated normalised CO_2 emission at time t ($y(t)$, in mg CO_2-C g^{-1} sample-C), which corresponds also to the fraction of mineralised C from each sample. First, the instantaneous CO_2-C flux (Φ_C, in mg CO_2-C min^{-1}) was calculated as the following equation:

$$\Phi_C = W \cdot X_{em} \cdot \frac{MM}{MV} \quad (7)$$

where MM is the molar mass of C (12.0107 g mol^{-1}), MV is the molar volume of an ideal gas at 20 °C (24.055 L mol^{-1}), W is the constant air flow (80 mL min^{-1}), and X_{em} is the

molar fraction of emitted CO_2 (in µmol mol^{-1}). Then, the instantaneous fluxes were cumulated and normalised by the total C amount in soil and substrate (sample-C) in each container:

(8) $$y(t) = \frac{1}{\text{Sample-C}} \cdot \sum_{t'<t} \Phi_C(t') \cdot \Delta t$$

where Δt = 2 hours is the interval between two subsequent measurements of the same sample and the summation is extended over all measuring time points from the beginning (t' = 0) to t' = t. The decay curves were subsequently fitted using a bi-exponential model as previously described by Cheng et al. (2008) and Liang et al. (2008):

(9) $$y(t) = Y_1 \cdot \left(1 - e^{-k_1 \cdot t}\right) + \left(1 - Y_1\right) \cdot \left(1 - e^{-k_2 \cdot t}\right)$$

where Y_1 (mg CO_2-C g^{-1} sample-C) and (1 – Y_1) are the fraction of the labile and of the recalcitrant C pool, and k_1 (d^{-1}) and k_2 ($year^{-1}$) are corresponding first-order kinetic constants. According to this model the decay velocity progressively decreases over time. The decay half-life ($t_{½}$, years) was calculated as $t_{½} = (\ln 2)/k_2$.

In some of samples an increase of the CO_2 emission was observed at a certain time point (t_p), which was define as "breakpoint" according to FOCUS (2006). In such cases an empirical model was applied, according to which the decay of C for $t > t_p$ continues with a different rate constant $k_3 > k_2$:

(10) $$y(t) = \begin{cases} Y_1 \cdot \left(1 - e^{-k_1 \cdot t}\right) + \left(1 - Y_1\right) \cdot \left(1 - e^{-k_2 \cdot t}\right) & \text{for} \quad t << t_p \\ Y_3 \cdot \left(1 - e^{-k_3 \cdot (t - t_p)}\right) & \text{for} \quad t >> t_p \end{cases}$$

where Y_3, the amplitude after the breakpoint, has been assumed to be independent from Y_1 and $(1 - Y_1)$. In that case two half-lives before ($t_{½}^{before}$) and after ($t_{½}^{after}$) the "breakpoint" were calculated, $t_{½}^{before} = (\ln 2)/k_2$ and $t_{½}^{after} = (\ln 2)/k_3$. The most suitable model for each sample was chosen comparing the plots of the residuals and the Akaike information criterion coefficient for all models.

4.2.5 Statistical analysis

An analysis of variance was conducted on the cumulated CO_2 emission after Day 2, 4, 6, 8 and 10, as well as on the fit parameters, to determine significant differences among the treatments within each experiment by means of the R software program version 3.0.2 (R core team, 2012).

4.3 Results

4.3.1 Soil respiration response to addition of chars

During the 10 days of incubation of the soil samples, a continuous emission of respired CO_2 could be observed in all treatments. The cumulated CO_2 emission for the soil alone (control), soil-straw mixture and all soil-char mixtures for various time points are listed in Table 4.2. The treatment with straw released the highest amount of CO_2, with a cumulative emission one order of magnitude higher than that of the control. Untreated pyrolysis char showed no significant difference in CO_2 emission compared to the control at any time. Respired CO_2 release from soil-digestate mixtures was significantly higher than the control or soil-pyrolysis char mixtures at any time. Respired CO_2 release in

presence of HTC char was always higher than that from pyrolysis char, and also higher than that in presence of digestate since day 6.

Table 4.2 – Cumulated CO_2 emission over time from different soil-substrate mixtures in the first incubation experiment. Means followed by the same letter(s) in the same column indicate that values were not significantly different at $P < 0.05$.

Treatment	Cumulated flux (mg CO_2-C g^{-1} sample-C)				
	2 days	4 days	6 days	8 days	10 days
Control	0.66 d	1.15 d	1.56 d	1.99 d	2.61 e
Straw	12.52 a	20.56 a	26.12 a	30.75 a	34.63 a
Pyro	1.13 d	1.68 d	2.11 d	2.62 d	2.93 e
HTC	2.19 bc	3.28 bc	4.95 b	7.41 b	9.74 b
Digestate	1.95 c	2.89 c	3.69 c	4.43 c	5.09 d
Pyro-ferm	1.83 c	2.66 c	3.28 c	3.90 c	4.60 d
HTC-ferm	2.57 b	3.90 b	5.06 b	6.24 b	7.14 c

4.3.2 Impact of fermentation post-processing

Fermented pyrolysis char induced a significantly higher CO_2 emission (nearly doubled) in comparison to untreated pyrolysis char. The CO_2 emission was slightly lower but not significantly different from the soil-digestate mixtures. In contrast, CO_2 emission in presence of fermented HTC char, despite being slightly, but not significantly higher than that of untreated HTC char, became significantly lower after Day 8, remaining approximately half-way between its components, the HTC char and the digestate.

Further information can be derived from a comparison of the emission data with the O:C and H:C molar ratios of the single substrates, obtained by elemental analysis (Table 4.1). The cumulated CO_2-C emission after 10 d correlated both with the H:C (R^2 = 0.77) and O:C (R^2 = 0.91) molar ratios (Figure 4.1).

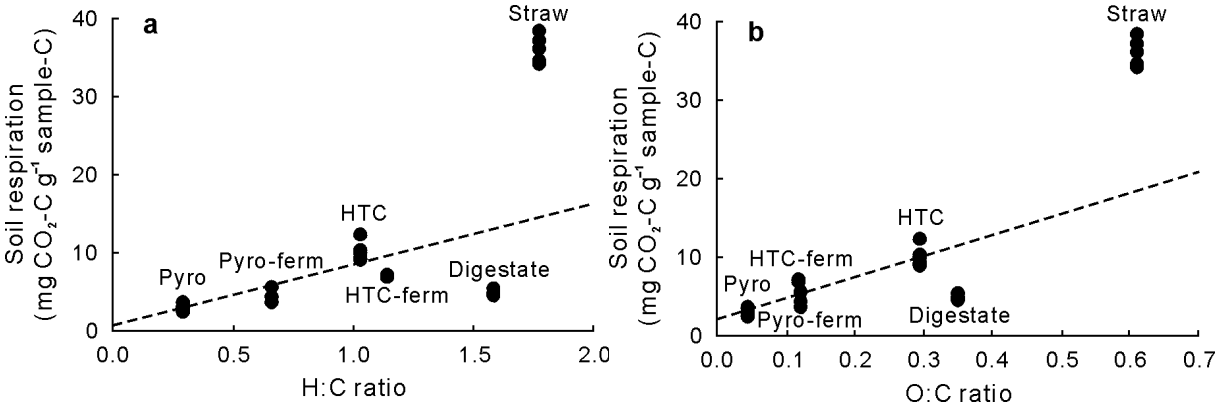

Figure 4.1 – Correlations between cumulated soil respiration and the H:C (a) and O:C (b) molar ratios of the substrates used in the first incubation experiment. The linear regression was calculated for chars only, excluding digestate and straw.

4.3.3 Impact of mineral N addition

Table 4.3 – Cumulated CO_2 emission over time from different soil-substrate mixtures, with or without mineral nitrogen, in the second incubation experiment. Means followed by the same letter(s) in the same column indicate that values were not significantly different at $P < 0.05$.

Treatment	Cumulated flux (mg CO_2-C g^{-1} sample-C)				
	2 days	4 days	6 days	8 days	10 days
Control	1.09e	1.88d	2.70d	3.59d	4.37c
Straw	13.35a	21.19a	27.14a	32.38a	37.28a
Pyro	1.38 de	1.96d	2.46d	2.92d	3.41c
HTC	2.44b	4.32b	7.34b	9.19b	10.94b
Mineral N	1.75cd	2.40d	3.00d	3.59d	4.25c
Pyro + N	1.31de	1.85d	2.28d	2.65d	3.02c
HTC + N	2.12bc	3.24 c	4.84c	7.18c	9.21b

The cumulated CO_2 emission for the soil and soil-char mixtures in the presence of mineral N is reported in Table 4.3. The addition of mineral N to soil alone induced a higher initial soil respiration, which became gradually negligible over the course of the incubation experiment. In the case of soil-HTC char mixtures, N addition resulted in a significantly higher emission only from Day 4 to Day 8 (Table 4.3): the CO_2 emission from HTC char in combination with mineral N appeared to be delayed in comparison to HTC char alone. Finally, in the case of pyrolysis char, addition of mineral N did not induce any significant differences in CO_2 release at any time during the incubation experiment (Table 4.3).

4.3.4 Dynamics of soil respiration

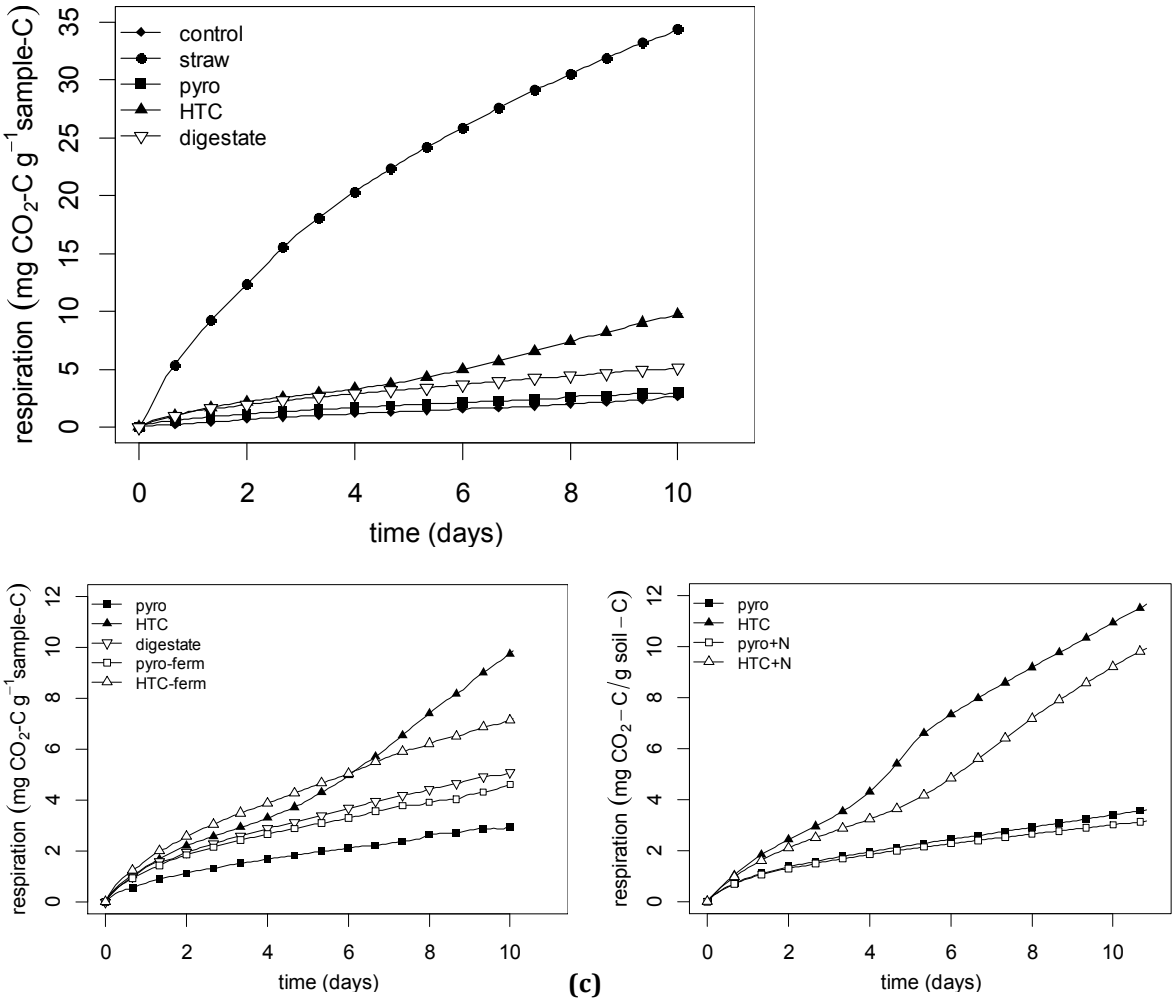

Figure 4.2 – Comparison of cumulated CO_2 emission over time from soil-substrate mixtures, normalised for the total C content of the mixture, between chars, straw and the control (a); between untreated and post-processed chars (b); and between the chars with added mineral N and the ones without (c).

The cumulated CO_2 emission over time from our soil-substrate mixtures is shown in Figure 4.2. In Figure 4.2a, the two chars are compared with the feedstock and the control soil; in Figure 4.2b, the post-processed chars are compared to the untreated ones, and in Figure 4.2c, the variants with mineral N are shown. All treatments, apart from HTC char and HTC char + N, showed a smooth increase of the mineralised C fraction, which slowed down with time. The treatments with HTC char, with or without mineral N, showed a

two-stage dynamics: After a comparably low activity in the first few days, a visible increase in the metabolic activity followed, starting on day 4.

Table 4.4 – Fit parameters of initial fraction of labile C (Y_1), kinetic constants (k_1, k_2 and k_3), and estimated half-lives ($t_{½}$) for different soil-substrate mixtures in the first and second incubation experiments. The fit parameters were obtained from the bi-exponential model for most mixtures and from the two-stage model for HTC and HTC + N. For the bi-exponential model, half life ($t_{½}$) was calculated; for the two-stage model, two half-lives before ($t_{½}^{before}$) and after ($t_{½}^{after}$) the "breakpoint" were calculated. Means followed by the same letter(s) in the same column for each experiment indicate that values were not significantly different at $P < 0.05$. The values of k_2 and k_3 were also checked for significant significancy against k_2 and the values for $t_{½}^{after}$ against $t_{½}^{before}$: for such comparisons, capital letters were used.

Substrate	Y_1 (mg CO_2-C g^{-1} sample-C)	k_1 (d^{-1})	k_2 ($years^{-1}$)	$t_{½}$ or $t_{½}^{before}$ (years)	k_3 ($years^{-1}$)	$t_{½}^{after}$ (years)
	First incubation experiment					
Control	0.48 b	1.15 a	0.07 b	10 a		
Straw	16.25 a	0.39 a	0.70 a	1 b		
Pyro	0.81 b	1.09 a	0.08 b	9 a		
HTC	1.33 b	5.61 a	0.28 bA	4 abA	0.37 A	2 A
Digestate	1.40 b	1.07 a	0.14 b	5 ab		
Pyro-ferm	1.48 b	1.00 a	0.11 b	6 ab		
HTC-ferm	1.72 b	1.02 a	0.20 b	3 b		
	Second incubation experiment					
Control	0.25 d	3.96 a	0.15 c	5 c		
Straw	12.92 a	0.53 b	0.92 a	1 e		
Pyro	0.99 c	1.19 ab	0.09 cd	8 b		
HTC	1.21 c	1.04 b	0.25 bA	3 dB	0.32 bA	2 B
Mineral N	1.19 c	1.40 ab	0.11 cd	6 c		
Pyro + N	1.10 c	1.00 b	0.07 d	10 a		
HTC + N	1.74 b	0.82 b	0.13 cdB	6 cA	0.30 bA	2 B

A bi-exponential fit of the emission curves yielded estimates for the parameters reported in Table 4.4. For all treatments except straw, the labile C pool Y_1 was found to be negligible in comparison to the total C mass (< 2 mg g^{-1}), being particularly low in the control and the HTC application. The kinetic constants lie in the range of days (k_1) and years (k_2 and k_3), respectively. No clear pattern appeared from k_1, while k_2 clearly had a maximum value for straw, significantly lower for all HTC treatments and lowest for all remaining treatments (digestate, mineral N and the control). For HTC char, the kinetic

constant after the "breakpoint", *i.e.* k_3, was not significantly different from the one before the "breakpoint", k_2, while for the combination of HTC char and N it was indeed higher. The estimated half-lives, related to k_2 and to k_3, ranged from about 1 year (straw) to about 10 years (the control), and the ones for chars fell between 2 years (HTC char) and 9 years (pyrolysis char).

4.4 Discussion

4.4.1 Soil respiration response and dynamics

Our initial hypothesis that soil respiration will decrease by the order of straw > HTC char > pyrolysis char > control was driven by considerations on the availability of easily degradable C compounds. The process of carbonisation (pyrolysis or HTC) is supposed to bind organic C compounds present in feedstock into more stable aromatic and thus more recalcitrant structures. Therefore, the respiration from a soil-char mixture was expected to be lower than for a soil-straw mixture, especially in case of pyrolysis char, which contained the highest amount of recalcitrant structures. This hypothesis was confirmed by our data, and is in accordance with previous reports (Bai et al., 2013; Qayyum et al., 2012). Furthermore, it was evident that substrates processed at higher temperatures showed lower H:C and O:C molar ratios and thus emitted less CO_2 in soil-char mixtures.

The substrates of this study showed a continuous emission flux decreasing progressively over time. Such emission curves are generally explained through a first-order decay kinetics involving one, two (Dicke et al., 2014; Gajić et al., 2012; Qayyum et al., 2012) or

more pools of degradable C compounds. A bi-exponential function usually fits the experimental data better than a mono-exponential function, especially if the dynamics embraces several time scales from days to centuries (Gajić et al., 2012; Qayyum et al., 2012; Zimmerman, 2010). The data in this study could be best fitted by a bi-exponential model, which points to the existence of at least two C pools, supporting our hypothesis that the heterogeneous composition of chars results in sequential stages of decomposition already detectable in short-term studies. The estimated half-lives of chars in this study are considerably shorter than those reported from other studies (Qayyum et al., 2012; B. P. Singh et al., 2012). This finding can be explained, on the one hand with the intrinsic uncertainty of the extrapolation, and on the other hand with the additional oxygen supply due to a permanent gas flow pumped through the samples in this experimental setup. However, for some of the substrates (HTC, HTC + N) the emission dynamics stood out from the others, showing a renewed increase of CO_2 emission after 4--5 d of incubation. Similar respiration kinetics were already described in microbiology as for instance in biogas research (Křivan, 2006; Schmidt et al., 1985) and also in the context of pesticide decay in soil (FOCUS, 2006), but to our knowledge this phenomenon has not been published in the context of biochar application to soil substrates before. The most common explanations proposed to describe such patterns are: (I) a lag phase, *i.e.*, soil microorganisms need time to adapt to a new substrate and the metabolisation rate increases gradually from low initial or basal levels to maximum values; (II) a sequential metabolism (diauxie), *i.e.*, soil microorganisms express initially a preference for some component(s) of the heterogeneous substrate until this component has been consumed and the population shifts, *i.e.*, it adapts its enzymatic apparatus to the mineralisation of other components; or (III) a simultaneous metabolism with

inhibition, *i.e.*, the metabolisation rates are initially lowered by some inhibiting agent present in the substrate, which is eventually metabolised or removed, after which the metabolism of the remaining substrate continues with enhanced velocity. All the cited sources actually describe a two-stage decay of a homogeneous substrate containing just one C pool (or two pools in the case of the diauxie), so they had to be modified to describe the kinetics of a highly inhomogeneous substrate like soil-HTC char mixture in this study. A rigorous implementation was not possible because of a too high number of highly correlated parameters. Therefore, we fitted our data with the empirical model described above. Thus, a relationship between the empirical fitting parameters, Y_3, k_1, k_2, k_3, and physically relevant parameters like growth rates or inhibition saturation constants can be deduced, making some mechanistic assumptions about the microbial metabolism. The occurrence of a multi-exponential dynamics demonstrates that at least two C pools play a role in soil respiration. It is clear that the emitted CO_2 can originate from two intrinsically heterogeneous sources, the char itself and the soil organic matter, which can also affect each other's dynamics through a positive or negative priming effect (Bamminger et al., 2014). The present study, however, was focused on the stability of the C compounds contained in the soil-char mixtures, which was estimated from the comparison between the cumulative emission and the elemental analysis of the samples. This seems reasonable as the stability of char has shown to correlate closely with the O:C molar ratio (Bai et al., 2014; Spokas, 2010). Accordingly, it was clearly evident that the two considered chars were more stable than the feedstock and that pyrolysis char was more stable than HTC char.

4.4.2 Impact of fermentation post-processing and mineral N addition

Respiration from soil amended with chars that had been post-processed by fermentation ranged in general between the one with untreated char and the one with digestate (Figure 4.2b). However, in the case of fermented pyrolysis char, the cumulated CO_2 emission was higher than for untreated char. This finding is in contrast to our hypothesis that fermented chars are always less biodegradable compared to chars without post-processing. Assuming that the fermentation post-processing does not decrease the stability of pyrolysis char, the increased soil respiration must be attributed to the digestate. In contrast, respiration from soil amended with fermented HTC char was reduced in comparison to unprocessed char (after day 4) and did not show the peculiar two-stage metabolism. One reason seems to be the composition of untreated HTC char, which is known to contain toxic compounds like phenols (Becker et al., 2013; Hale et al., 2012), which hold the potential to inhibit microbial activity and thus respired CO_2. Since no inhibition was observed from soil mixed with fermented HTC char, this finding suggests that, in the case of HTC char, the fermentation is effective in both reducing respired CO_2 and removing toxic compounds.

The different substrate qualities of pyrolysis and HTC chars could be partly explained by different chemical composition and structure (Figure 4.1), and furthermore by interactions with digestate components during post-processing. According to Spokas (2010), the O:C molar ratio is a good indicator of char stability: that implies that fermented HTC is a more stable product than the raw mixture, and in particular it is more stable than HTC char alone. Furthermore, the elemental abundances of the post-processed chars can be related to the composition of the corresponding untreated char-

digestate mixtures. Fermented pyrolysis char has H:C and O:C ratios (H:C = 0.66, O:C = 0.12; Table 4.1) scarcely different from an untreated pyrolysis char-digestate mixture with a mass ratio 2:1 (H:C = 0.55, O:C = 0.11); therefore it behaves like an untreated mixture and, in particular, it induces respired CO_2 located between those of the two components. The O:C ratio for fermented HTC char (0.12) was much lower in comparison to an untreated HTC char-digestate mixture (0.31), signalling that the co-fermentation removed part of the oxygen present in the two components; consequently, the transformed product induced a lower CO_2 release than a simple mixture of the two components and can therefore be assumed to have a higher stability.

The addition of mineral N did not exert an influence on the total CO_2 released and, thus, the hypothesis that mineral N applied to soil mixed with HTC and pyrolysis chars will increase soil respiration was not confirmed. It can be argued that the added N is adsorbed or chemically bound by the functional groups on the char surface, thereby cancelling the effect of the additional nutrients. However, several effects on the soil respiration kinetics were observed, i.e., in the absence of chars, the CO_2 emission increased during the first few days, but was delayed in the presence of HTC char, or was not affected at all in the soil mixed with pyrolysis char. These apparently contrasting effects must be explained carefully. In the control soil, the addition of mineral N decreased the C:N ratio of the microenvironment and thus stimulated microbial activity, until available N was consumed. Pyrolysis char is known to absorb cationic compounds present in the soil (Liang et al., 2006), which was apparently effective in this study to immobilize the fertiliser addition. In the case of HTC char, it is known that toxic compounds may occur (Hale et al., 2012), thus it can be assumed that transformation by

soil microorganisms may yield more toxic compounds, resulting in a negative feedback on the activity of the microorganisms themselves, but further studies would be required to resolve these findings.

4.5 Conclusions

This short-term dynamic incubation study provided evidence that all conversion products of maize straw were less readily decomposed than the straw itself. The digestate and the chars from pyrolysis and HTC were stabilised compared to the original straw substrate. All conversion processes under this study, pyrolysis, HTC and fermentation could thereby contribute to mitigate the emission of CO_2. However, the type of carbonisation and post-treatment were responsible for different respiration kinetics.

In most cases, the respiration kinetics followed a bi-exponential trend over the study period, pointing to a heterogeneous composition of the chars. An interesting finding was the two decomposition stages observed in the respiration dynamic of untreated HTC char, which can be explained by an initial inhibition effect, probably triggered by toxic compounds. The mechanisms responsible for degradation of toxic compounds and the adaptation of microorganisms are not yet known, but they will further open promising studies on the interaction between biochar and soil biota.

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5. Impact of chars and readily available carbon on soil microbial respiration and microbial community composition in a dynamic incubation experiment

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Abstract

The carbonisation of biomass and organic residues is discussed as an opportunity to store stabilised carbon compounds in soil and to reduce mineralisation and the emission of CO_2. In this study, pyrolysis char (600 °C, 30 min) and hydrothermal carbonisation char (HTC char; 210 °C, 23 bar, 8 hours), both derived from maize silage, were investigated in a short-term incubation experiment of soil mixtures with or without readily available carbon (glucose) in order to reveal impacts on soil microbial respiration and community composition. In contrast to pyrolysis char, the addition of HTC char increased respiration and enhanced the growth of fungi. The addition of glucose to soil-char mixtures containing either pyrolysis or HTC char induced an

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additional increase of respiration, but was 35 % and 39 % lower compared to soil-glucose mixtures, respectively, providing evidence for a negative priming effect. No significant difference was observed comparing the soil mixtures containing pyrolysis char + glucose and HTC char + glucose. The addition of glucose stimulated the growth of most microbial taxa under study, especially of Actinobacteria at the expense of fungi. Adding pyrolysis or HTC char to soil induced a decline of all microbial taxa but did not modify the microbial community structure significantly. Addition of pyrolysis or HTC char in combination with glucose however, increased the abundance of Actinobacteria and reduced the relative abundance of Acidobacteria and Betaproteobacteria while fungi were further increased in case of HTC char. We conclude that both chars hold the potential to bring about specific impacts on soil microbial activities and microbial community structure, and that they may compensate the variations induced by the addition of readily available carbon.

Keywords

carbon turnover, HTC char, microbial communities, pyrolysis char, short-term study

5.1 Introduction

Char materials, which derive from thermochemical carbonisation of biomass, have been proposed as one option for long-term carbon storage and for the improvement of soil properties (Lehmann et al., 2006). The two main processes studied in recent years are pyrolysis and hydrothermal carbonisation (HTC), besides other techniques such as vapothermal carbonisation (Funke et al., 2013), gasification and fast pyrolysis (Libra et

al., 2011). In contrast to pyrolysis, which is a dry process running under anaerobic conditions at temperatures between 200 °C and 900 °C (Lehmann et al., 2006), HTC is performed in aqueous systems under autogenous pressure of about 10 to 20 bar at temperatures between 180 °C and 250 °C (Libra et al., 2011). According to the different process conditions, the products defined as pyrolysis char and HTC char, have completely different properties. Compared to pyrolysis chars, HTC chars have a lower carbon content and correspondingly higher contents of hydrogen and oxygen due to their lower carbonisation degree. The relationship between the carbon content of the char material and its stability against microbial decay has been described manifold (Bai et al., 2013; Busch and Glaser, 2015; B. P. Singh et al., 2012; Spokas, 2010).

The application of biochar, or carbonised organic matter to soils has been proposed as a method for the long-term storage of organic carbon in the environment, which at the same time will provide agronomic benefits due to the improvement of soil properties (Lehmann et al., 2006; Schulz and Glaser, 2012). Biochar in soil can increase the stability of soil aggregates and the availability of nutrients, which in turn have positive effects on plant growth and biomass yields (Biederman and Harpole, 2013; Lehmann et al., 2006). Moreover, variable effects on the abundance and composition of soil fauna and microflora were described, depending on environmental conditions (Atkinson et al., 2010; Lehmann et al., 2011). The extent, or the duration of the impacts of biochar is strongly dependent on its degradability, which results from complex biochemical mechanisms, which in turn depend on several external factors in the respective ecosystem, such as the physico-chemical and climatic conditions; the amount, quality and availability of carbon; the availability of nutrients and energy sources; and the

microbial abundance and activity and the influence of mycorrhiza and soil fauna. In order to understand the mechanisms of char degradation, and therefore the feasibility of its application in a soil amendment system, an inspection of its effects on soil microbial activity and community composition is crucial. Microbial activity can be assessed under defined experimental conditions by approaches based on respiration (Blagodatskaya and Kuzyakov, 2013; Lanza et al., 2015), while microbial community composition can be studied by DNA sequencing techniques such as qPCR. Chars have shown to have several direct and indirect effects onto microbial communities. Addition of biochar to top soil may stimulate the activity of soil bacteria and fungi already on a short time scale (Ameloot et al., 2013; Bamminger et al., 2014), especially under stressful environmental conditions like during water scarcity (Liang et al., 2014). In a previous investigation with the same char materials as used in this study in presence of nitrogen fertiliser (Lanza et al., 2015) we did not find a significant response in soil respiration upon addition of pyrolysis char, but a significant increase upon addition of HTC char deriving from the same substrate (maize silage). Microbial community composition is also affected; recent studies reported an overall increase of various taxa of microorganisms after biochar addition to soil, such as Gram-positive and Gram-negative bacteria (Ameloot et al., 2013), Actinobacteria (Prayogo et al., 2014), or fungi (Steinbeiss et al., 2009), though in some cases growth was reduced during the first weeks (Mitchell et al., 2015) and the reaction differed depending on soil types (Chen et al., 2015). Several mechanisms behind impacts of biochars on the soil microflora were summarised and changes in microbial activity or community structure explained (Thies et al., 2015). Biochar may provide habitat or shelter for soil organisms (Quilliam et al., 2013) and promote soil ecological conditions, such as water holding capacity or buffer capacity (Karhu et al.,

2011). Moreover, biochar may be source of energy (Watzinger et al., 2014) and nutrients (Warnock et al., 2007) and thus it may interact with soil trophic chains in the soil-plant system (McCormack et al., 2013).

In general, the addition of readily available organic matter to soil has shown to increase microbial activity and also to induce changes in the microbial community composition (Cleveland et al., 2007). However, simultaneous addition of chars and readily available organic carbon sources can lead to interaction effects on soil community composition, as well as modification of the degradability of both additives, so called priming effects (Kuzyakov, 2010). Both positive (Hamer et al., 2004; Jones et al., 2011) and negative (Whitman et al., 2014) priming effects of chars on the decay of soil organic matter have been reported and discussed (Kuzyakov, 2010; Woolf and Lehmann, 2012). Even in some cases, the priming was either positive or negative at different points in the course of time (Maestrini et al., 2014). Against this background we performed incubation experiments with chars and glucose, intending to amplify any char-induced impacts and to inspect possible interactions between these two different carbon sources in terms of availability.

According to a previous study (Lanza et al., 2015), pyrolysis char and HTC char made from the same feedstock, i.e. maize silage, were tested in a 10 day incubation besides the feedstock itself and a soil control without any substrate addition. The aims of the present study were to test the following hypotheses:

(1) Chars, being mostly inert material, do not impact overall soil microbial activity and microbial abundance;

(2) Different chars promote differences in soil microbial respiration and shifts in microbial community composition;

(3) Addition of a readily available carbon source to soil-char mixtures promotes additional soil respiration and shifts in microbial community composition.

5.2 Materials and methods

5.2.1 Preparation of the chars

Maize straw samples were taken from an experimental field site located in Braunschweig, Germany (Becker et al., 2014), ground in an ultra-centrifugal mill (0.75-mm sieve) and stored until used. All other substrates tested in our study were produced from maize silage. Pyrolysis char (REW, Quakenbrück, Germany) was produced in a continuous reactor (600 °C, 30 min) and quenched by means of water sprinkling. HTC char (AVA CO_2, Karlsruhe, Germany) was produced in a one-pot batch reactor (210 °C, 23 bar, 8 hours) and separated by means of a chamber filter press. After production, all chars were stored at -20 °C. A few weeks before the experiments started, the samples were unfrozen, oven-dried for 48 h at 105 °C, ground up to a fine powder and stored at 4 °C. The pH value of straw and chars was measured 1:5 in distilled water. The straw and the carbonised products were analysed for total C and N content with an elemental analyser (Vario EL III, Elementar, Germany).

The chemical properties of the substrates used are listed in Table 5.1.

Table 5.1 – Physico-chemical properties of the substrates used.

Substrate	pH	DM	oDM	C	N
		(mg g^{-1} FM)	(mg g^{-1} DM)	(mg g^{-1} DM)	(mg g^{-1} DM)
Soil	4.72	929	14.7	6.26	0.55
Straw	6.29	939	926	464	14
Pyro	9.72	973	837	756	17
HTC	5.18	984	966	636	23

5.2.2 Preparation of soil-char mixtures

The soil used was taken from the top layer (0-15 cm) of an experimental field located in Berge (Kreis Havelland, Brandenburg, Germany, 52° 63′N, 12° 80′E), which represents a typical site of the glacial landscape of North-eastern Germany. It was a loamy sand (Haplic Cambisol) with the following texture: 712 mg g^{-1} sand (Ø > 630 µm), 222 mg g^{-1} silt (2-630 µm) and 66 mg g^{-1} clay (Ø < 2 µm). The chemical properties of the soil are also included in Table 5.1.

The field-moist soil (dry mass = 93 %) was sieved up to a particle size < 2 mm and stored at 4 °C in a container until analysis. After equilibration (2 d, 20 °C), soil was mixed with either straw meal or char (5 mg DM g^{-1} soil, corresponding to 2 to 4 mg C g^{-1} soil) using a kitchen mixer. D(+)-glucose, anhydrous (Merck, Germany) was added to half of the samples also in the amount 5 mg DM g^{-1} soil, corresponding to 2 mg glucose-C g^{-1} soil.

5.2.3 Incubation design and CO_2 measurement

Soil-substrate mixtures (100.5 g FM per sample) were incubated in three replicates in Plexiglas tubes (4 cm diameter) for 240 h at 20 °C at constant soil moisture (75 mg H_2O

g^{-1} DM), using an automated system for continuous soil respiration measurements (Heinemeyer et al., 1989). The molar fraction of the emitted CO_2 (X, in ppm) was measured in a continuous flow of W = 80 ml min^{-1} with a periodicity Δt = 2 hours by using a Picarro G1101-i analyser (Picarro Inc., CA, USA) connected to the system via a T-pipe.

5.2.4 Extraction of DNA

For each treatment, one aliquot of each soil mixture was collected before onset of the incubation experiment and three (one per each replicate) at the end of the experiment.

Samples were thereafter stored at -20 °C until extraction of total genomic soil DNA using the NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). For resulting DNA samples, DNA concentration and nucleic acid purity was assessed using NanoDrop 2000 Spectrophotometer (NanoDrop products, Wilmington, DE, USA) and nucleic acid agarose gel electrophoresis. DNA Samples were subsequently stored at 4 °C for further processing.

5.2.5 Amplification of DNA

qPCR (quantitative Polymerase Chain Reaction) assays were conducted in polypropylene 96-well plates on a QuantStudio™ 12K Flex Real-Time PCR System (Life Technologies, Grand Island, NY, USA). Seven different primer pairs of taxa specific genes were separately used to quantify abundance of fungi and the bacterial taxa Firmicutes, Actinobacteria, Acidobacteria, Alpha-, Beta- and Gammaproteobacteria. The primers and corresponding standard microorganisms were chosen in agreement to Fierer et al.

(2005). Besides the unknown samples, each plate included the appropriate standards in a 10fold dilution series (from 10^{-1} to 10^{-7} in 3-fold replicate) to generate standard curves, as well as negative and positive controls. Each 20 µl reaction well contained: 4 µl of 5× HOT FIREPol® EvaGreen® HRM Mix ROX (Solis Biodyne, Tartu, Estonia), 0.25 µl of each primer (10 pM, biomers.net), 14.5 µl of Millipore H_2O and 1 µl of template DNA. The performed runs consisted of an initial denaturation phase (15 min at 95 °C), followed by 40 amplification cycles (15 s at 95 °C, 20 s at 60 °C and 30 s at 72 °C). The progress of the amplification was tracked by means of an integrated optical detector which measured the fluorescence signal from the complete double strands over time. The quality of each run was assessed through a melting curve analysis of the PCR products.

5.2.6 Data analysis and statistics

The soil respiration response was quantified as cumulated CO_2 flux ($y(t)$, in mg CO_2-C g^{-1} soil) from each sample. First, the instantaneous CO_2-C flux (Φ_C, in mg CO_2-C min^{-1}) was calculated after correcting the CO_2 molar fraction by subtraction of a background:

$$\Phi_C = W \cdot \left(X - X_{bg}\right) \cdot \frac{MM}{VM} \tag{11}$$

where MM = 12.0107 g mol^{-1} is the molar mass of carbon and VM = 24.055 L mol^{-1} is the molar volume of an ideal gas at 20 °C. Then the fluxes were cumulated and normalised by the total carbon amount (soil + substrate) in each container:

$$y(t) = \frac{1}{C_{tot}} \cdot \sum_{t'<t} \Phi_C(t') \cdot \Delta t \tag{12}$$

where $t' = 0, \Delta t, 2 \cdot \Delta t, \ldots t$. An analysis of variance, followed by a Tukey test at significance level $\alpha = 0.05$, was conducted on the cumulated fluxes after day 2, 4, 6, 8 and 10 to determine significant differences among the treatments within each experiment by means of the software R, version 3.0.2 (R core team, 2012).

The total DNA in each sample was calculated on basis of the extracted DNA concentration reported by measurement of optical absorbance (Nanodrop, NanoDrop products, Wilmington, DE, USA), in ng μl^{-1}. The abundance of each microbial taxon in each sample was quantified as the DNA amount of the corresponding gene (in ng μl^{-1}), quantified by qPCR, obtained from the *Ct* value of the corresponding amplification curve. A variance analysis was performed on the average values by means of the software STATISTICA 10.

5.3 Results

5.3.1 Soil respiration response

During the incubation, a continuous emission of CO_2 was observed in all treatments. The cumulated CO_2 release at various time points is listed in Table 5.2. The maximum CO_2 release was induced by glucose, which at the end of the experiment (Day 10) was 2.4 times as high as compared to straw meal. Both soil-char mixtures emitted significantly less CO_2 compared to the soil-straw mixture (Figure 5.1). The release of CO_2 from the treatment with pyrolysis char did not differ significantly from the control while CO_2 release from the HTC treatment was significantly higher. The combined addition of char (either pyrolysis or HTC) and glucose significantly increased CO_2 release but no

difference was observed comparing both char treatments. In comparison to the glucose treatment, the combination of char (either pyrolysis or HTC) and glucose reduced soil CO_2 release between 35 % and 39 %, almost constantly over time.

Table 5.2 – Cumulated respiration over time for the chars with and without glucose. Values followed by the same letter(s) in the same column indicate are not significantly different at $P < 0.05$.

Treatment	Cumulated flux (mg CO_2-C g^{-1} sample-C)				
	2 days	4 days	6 days	8 days	10 days
Control	0.50 e	0.91 e	1.29 e	1.79 f	2.31 e
Straw	10.86 c	21.47 c	27.41 c	31.76 d	35.41 c
Pyro	0.87 e	1.37 e	1.8 e1	2.20 f	2.56 e
HTC	1.66 d	3.19 d	4.81 d	6.26 e	7.81 d
Glucose	19.57 a	41.26 a	59.78 a	75.49 a	85.93 a
Pyro+Gluc	12.61 b	26.13 b	37.71 b	48.31 b	55.70 b
HTC+Gluc	12.46 b	25.58 b	36.59 b	46.02 c	53.77 b

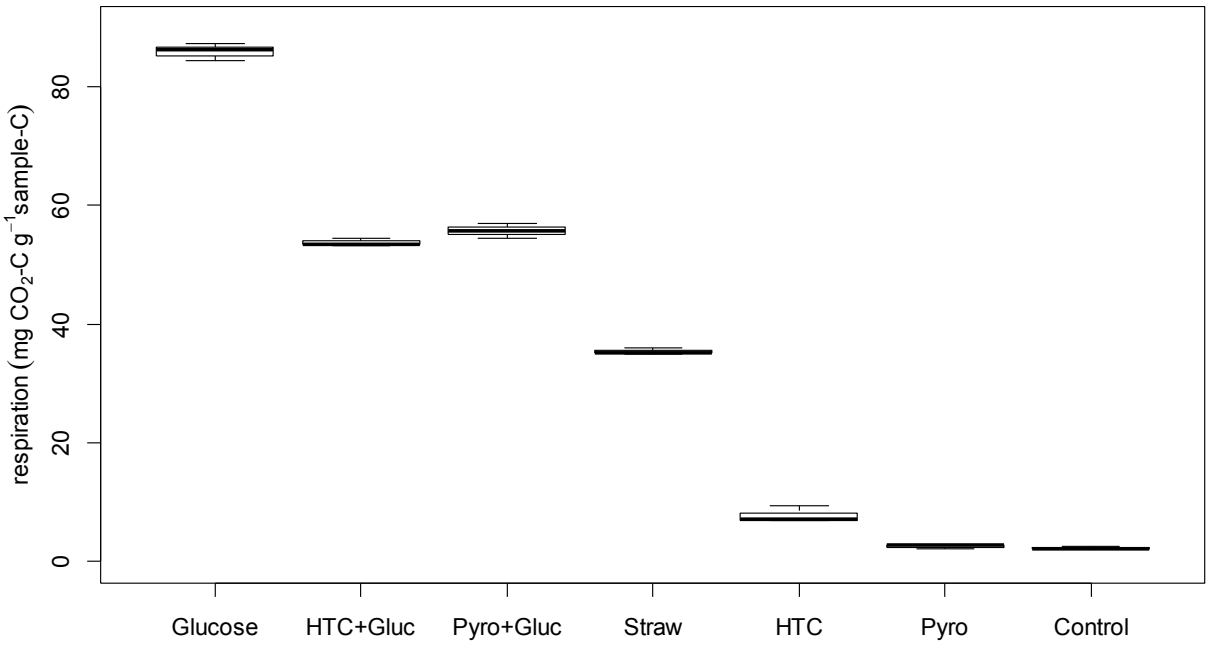

Figure 5.1 – Cumulated CO_2 release in control soil and soil-char mixtures, with or without glucose.

5.3.2 Soil microbial community dynamics

The total soil DNA content in the extracted solutions was in a range between 90 ng μl^{-1} (in case of HTC treatment) and 140 ng μl^{-1} (in case of straw treatment), with no significant differences between all the treatments (data not shown). The incubation of the control soil induced an increased abundance for all microbial taxa under study, especially for Actinobacteria, whereas the addition of both chars induced a decrease in the abundance of all taxa under study (Table 5.3). The treatment with glucose, however, clearly increased the growth of Gammaproteobacteria, Actinobacteria, Alphaproteobacteria and Firmicutes and suppressed Acidobacteria and Betaproteobacteria. The combination of pyrolysis char with glucose enhanced the growth of Gammaproteobacteria, Actinobacteria, Alphaproteobacteria and Firmicutes and suppressed Acidobacteria. The combination of HTC char with glucose also greatly enhanced Gammaproteobacteria and decreased Acidobacteria in a similar extent to pyrolysis char, but in contrast clearly slowed down Betaproteobacteria, Acidobacteria and Firmicutes and stimulated greatly fungi.

Table 5.3 – Dynamics of microbial taxa, expressed as relative changes to the corresponding initial values of each variant (in %).

Treatment	Population dynamics (±%)						
	Actino	Acido	Alpha	Beta	Gamma	Firmi	Fungi
Control	43.0	2.5	10.1	16.0	1.8	25.6	8.8
Straw	71.5	-12.7	31.2	14.2	46.9	27.5	-20.1
Pyro	-8.1	-10.5	-15.4	-2.3	-16.9	-17.7	-31.0
HTC	-25.5	-37.5	-5.7	-12.5	-23.3	-38.4	-7.3
Glucose	68.6	-43.2	23.9	-15.0	158.8	60.9	2.3
Pyro+Gluc	96.0	-38.4	36.8	-3.8	187.1	45.0	7.6
HTC+Gluc	17.3	-44.2	-4.8	-44.6	156.7	-51.5	60.0

Based on these dynamics, a similarity analysis separated two major groups, cluster (I) including the variants control, straw and pyrolysis char and cluster (II) including all three variants with glucose addition while the HTC treatment was isolated, closer to the glucose variants (Figure 5.2).

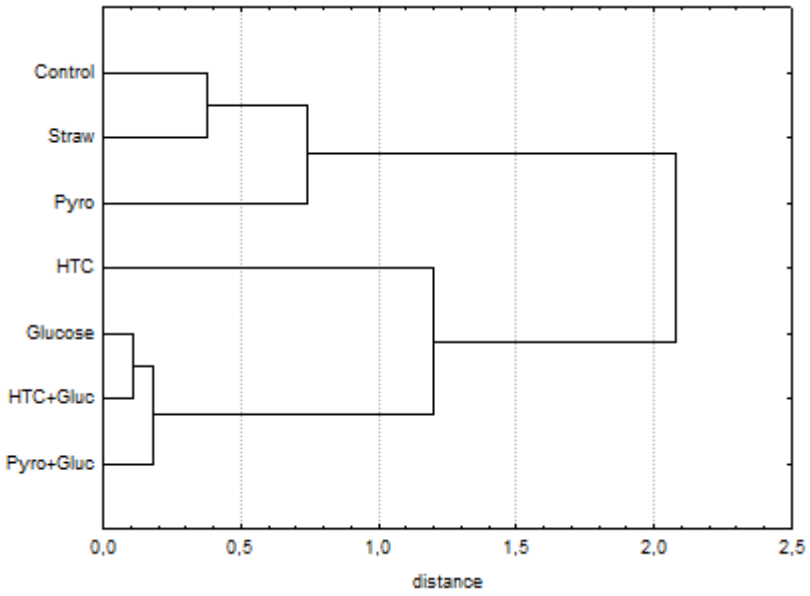

Figure 5.2 – Cluster analysis based on the dynamics of microbial taxa in the treatments (see Table 5.3).

5.3.3 Abundance of microbial taxa and community structure

Significant differences in microbial community among the variants were found at the end of the incubation experiment (Table 5.4). Compared to the control, the addition of either straw or glucose resulted in a higher abundance of Actinobacteria and a lower abundance of fungi by tendency. Pyrolysis char alone induced no significant changes of the taxa under study, while HTC char tended to increase the abundance of fungi, but only in combination with glucose. Both chars combined with glucose reduced the abundance of Acidobacteria and increased Gammaproteobacteria significantly. Alphaproteobacteria

and Gammaproteobacteria were significantly reduced in the HTC + glucose treatment, what was not found for pyrolysis char.

Table 5.4 – Relative abundance of microbial taxa at Day 10 of the incubation (in %). Values followed by the same letter(s) in the same column indicate are not significantly different at $P < 0.05$.

Treatment	Relative abundance (%)						
	Actino	Acido	Alpha	Beta	Gamma	Firmi	Fungi
Control	8.8 a	8.7 bc	2.7 ab	8.3 ab	0.4 a	0.2 ab	70.9 ab
Straw	14.7 b	7.7 bc	2.9 bc	9.5 b	0.7 a	0.3 b	64.0 a
Pyro	9.3 a	9.2 c	2.5 ab	8.0 ab	0.4 a	0.2 ab	70.4 ab
HTC	8.9 a	6.6 ab	2.8 ab	8.3 ab	0.4 a	0.1 a	72.8 b
Glucose	17.2 b	6.8 ab	3.5 c	8.1 ab	1.9 c	0.2 ab	62.3 a
Pyro+Gluc	14.7 b	4.9 a	2.9 bc	6.1 a	1.5 bc	0.2 ab	69.5 ab
HTC+Gluc	10.9 a	4.9 a	2.2 a	5.9 a	1.3 b	0.1 a	74.6 b

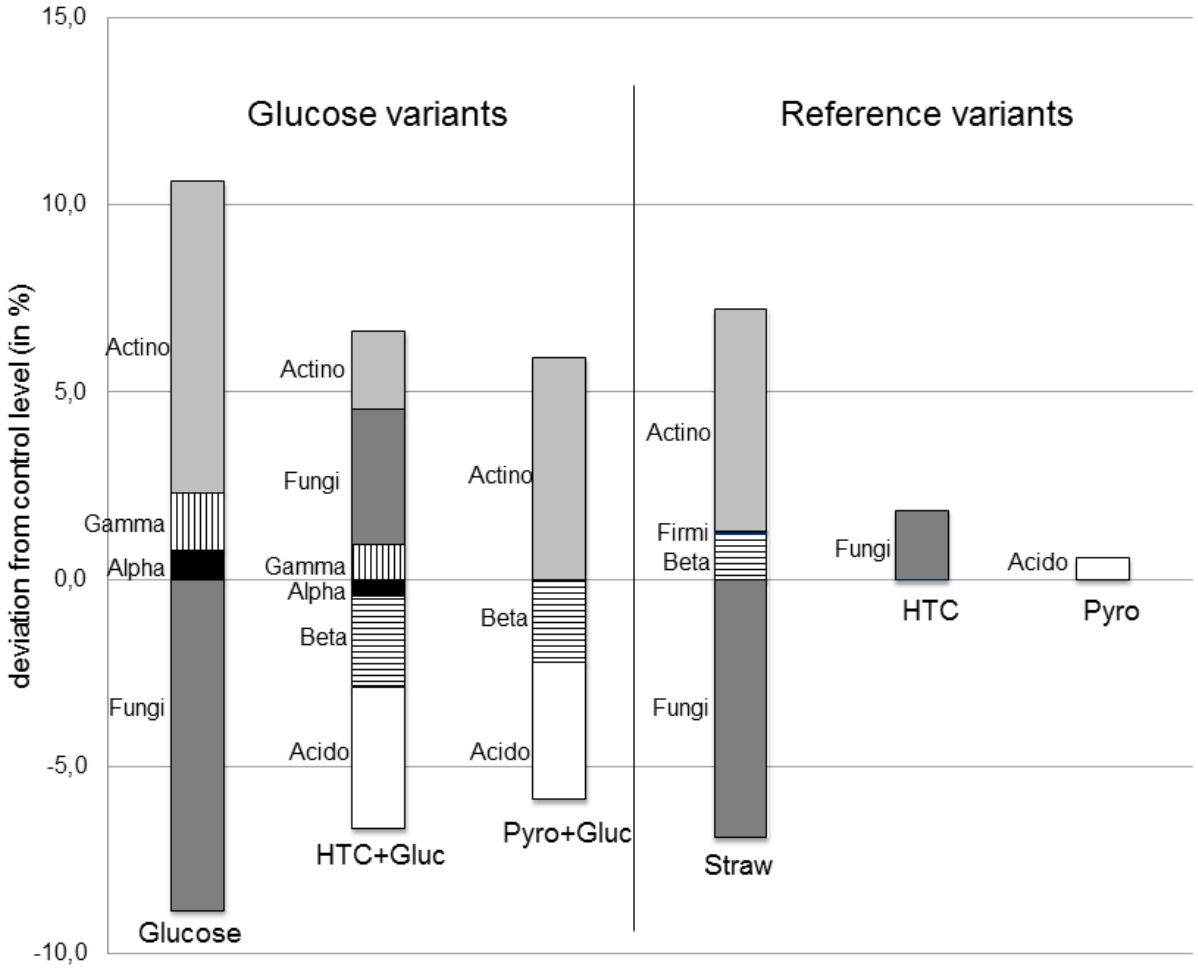

Figure 5.3 – Differences in the relative abundance of microbial taxa, referred to the control, after 10 days of soil incubation (in %).

Changes in community structure after incubation are shown in Figure 5.3, expressed as relative abundance of microbial taxa, referred to the control. The addition of glucose and straw decreased the relative portion of fungi, but fungi were enhanced after addition of HTC or HTC + glucose. In the variants combining char and glucose, the relative proportions of Acidobacteria and Betaproteobacteria were decreased, as well as the proportion of Alphaproteobacteria in the HTC + glucose treatment.

5.4 Discussion

5.4.1 Effects of chars on soil respiration and microbial community composition

During our short-term incubation study, the addition of pyrolysis char had no impact on soil respiration, whereas the addition of HTC char increased soil respiration, as reported previously (Bai et al., 2013; Lanza et al., 2015). This finding can be explained by the different amounts of recalcitrant structures and is in accordance to other reports, e.g. (Bai et al., 2013). Furthermore, (Mitchell et al., 2015) reported that initially unfavourable changes in microbial habitat or the introduction of compounds associated with biochar such as polycyclic aromatic hydrocarbons, residual pyrolysis oils and polar pyrolysis condensates may be the reason for toxic effects on microorganisms and their activity (Hale et al., 2012; Spokas et al., 2011). A trend of decreased microbial biomass in soils amended with biochars produced from feedstocks with high lignocellulosic content has been reported by (Gul et al., 2015). This finding corresponds to a previous study of (Gomez et al., 2014) who reported concentration-dependent changes in microbial activity in response to biochar addition but without major changes in community

composition at the lowest application rates, which were even higher, compared to concentrations used in our study.

The abundance of all microbial taxa under study did not significantly differ after the addition of chars in our short-term incubation experiment. However, the analysis of community structure indicated an enhanced proportion of fungi after HTC treatment in relation to the control but, with respect to the significantly increased respiration, this change is considered to be just a hint for structural changes within this taxon. An increased growth of the fungi after char addition and a change in their proportion of the total community is in agreement with other publications (Steinbeiss et al., 2009; Titirici et al., 2012) and may be explained by a benefit for fungal proliferation in nutrient poor and acidic environments, especially in the presence of volatile organic carbon compounds such as furfurals, phenols and also organic acids, which are known to be sorbed on HTC chars (Hale et al., 2012; Spokas et al., 2011). These compounds may undergo volatilisation and decomposition in the course of time, due to the extracellular enzymatic activity of fungi (Nichols et al., 2008). Similar processes obviously do not occur for pyrolysis char, which is carbonised to a higher degree and which furthermore may induce an increase in soil pH (Cayuela et al., 2014). Such conditions are known to be detrimental for fungal growth (Gul et al., 2015). At the beginning of the incubation experiment, microbial abundance tends to be higher in the char variants; thus the dynamics of all microbial taxa towards the endpoint followed a negative trend, matching more or less the control levels, except for fungi in the HTC treatment. In contrast to the chars, a completely different reaction of the microbial community was detected when straw was applied to soil, especially with respect to the Actinobacteria, which gained a

predominant role at the expense of fungi. These bacteria are well-known for their capacity to metabolise recalcitrant substrates such as ligno-cellulose (Jiang et al., 2016; McCarthy and Williams, 1992), which makes up the main straw component.

5.4.2 Combined effects of glucose and chars on soil respiration and microbial community

Addition of glucose as a readily available carbon source to soil-char mixtures induced an additional increase of respiration and promoted evident changes in microbial community structure. The respiration response upon glucose addition was similar for both chars, indicating that char-derived carbon did not play the major role as a carbon substrate for microbial activity, although differences were detected comparing pyrolysis and HTC char without glucose addition.

However, compared to the respiration response to glucose addition in the absence of chars, the presence of either char remarkably reduced glucose respiration in soil, as well as the amplitude of variation in the relative abundance of microbial taxa, particularly of Acidobacteria, Betaproteobacteria, Gammaproteobacteria and fungi. Based on these findings, the chars are considered to have exerted a negative priming effect. Since HTC char is known to have a lower stability compared to pyrolysis char (Lanza et al., 2015), the over-all respiration in absence of a priming effect should be higher for HTC char + glucose compared to pyrolysis char + glucose. However, there was no significant difference between both treatments and it can be assumed that HTC char exerts a higher negative priming effect than pyrolysis char, which is in agreement with recent studies using chars from the same feedstock (Bamminger et al., 2014; Malghani et al., 2013). In

order to differentiate and quantify the mineralisation of distinct carbon sources (glucose, char, or soil organic carbon), measurements of the isotopic composition of CO_2-C would be required as shown by (Kuzyakov et al., 2009) by using ^{14}C-enriched pyrolysis char derived from ryegrass. The authors reported a glucose-induced increase in soil respiration in a similar extent compared to our results and calculated a char decomposition rate of 0.5 % per year.

With respect to soil microbial communities, the abundances of Actinobacteria, Alphaproteobacteria and Gammaproteobacteria at the end of the experiment were increased in all treatments with a high respiration rate (straw, glucose), while fungi showed a reversed tendency. A correlation analysis between respiration and DNA abundance of the single taxa yielded a high correlation coefficient for the abundance of Gammaproteobacteria (R^2=0.95) and Actinobacteria (R^2=0.71), confirming that these taxa play an important role in the degradation of soil organic carbon compounds. The decreased glucose-induced respiration response in the presence of chars does not correspond to a general decline of microbial taxa, but is considered an adaptation effect which is specific for each char, but nevertheless results in the same soil respiration activity. For both chars, the relative abundance of Betaproteobacteria and Acidobacteria was reduced and the relative abundance of fungi, which was declined by glucose, was restored in case of HTC addition. In general, our results show that the main effect of both chars with respect to microbial communities is manifested in taxa-specific abundance and structure, and furthermore these effects are char-specific.

In more detail, the adaptation of Betaproteobacteria spans from a significant enhancement after addition of straw to a striking reduction after the addition of chars +

glucose. It was shown previously (Parales, 2010) that Betaproteobacteria play an important role in the degradation of aromatic hydrocarbons, also (Eilers et al., 2010) reported a significant dominance of Betaproteobacteria in a coniferous soil, which could explain an adaptation to recalcitrant organic carbon compounds. The decrease of the common soil taxon Acidobacteria (Dunbar et al., 2002) was significantly enhanced by pyrolysis char and was reduced in all other treatments, particularly after the addition of both chars in combination with glucose, which seems to be not only an adaptation to soil pH-values (Kishimoto et al., 1991), but rather an adaptation to more oligotrophic conditions (Eichorst et al., 2007; Koch et al., 2008) after addition of char. The reduction of Acidobacteria abundance by about one half is also seen in bulk Terra Preta soils characterised by highly increased amounts of stabilised organic compounds, charcoal, bone, and pottery sheds as compared to the corresponding non-anthropogenic adjacent soil (Barbosa Lima et al., 2015). The abundance of bacterial taxa that preferred nutrient-rich environments, such as Actinobacteria, showed a similar trend after the addition of straw or glucose as found in a field study about adding maize residues (Ramirez-Villanueva et al., 2015). Surprisingly, the treatment HTC + glucose with a per saldo similar nutrient level as the treatment without HTC char reduced the abundance of Actinobacteria significantly in favour of fungi, which are best adapted to low-pH conditions and to the possible occurrence of volatile organic carbon compounds, thus suppressing bacteria. Further studies are required to resolve such dynamics, both in the short and the long term.

5.5 Conclusions

Our study showed that the addition of chars, especially in the presence of readily available carbon, modifies soil conditions in terms of microbial respiration response and microbial community composition. In contrast to pyrolysis char, the addition of HTC char stimulated microbial activity and enhanced the growth of fungi. Upon addition of chars to a system enriched with glucose, respiration rates were significantly reduced and shifts in microbial community composition were detected. We conclude that chars hold the potential to bring about specific impacts on soil microbial activities and microbial community structure already in the short term, and may compensate or counteract the variations induced by the addition of readily available carbon. Thus the decision to use biochar as a soil amendment must carefully weigh the proposed benefits such as an increased nutrient availability or carbon sequestration potential against non-predictable changes in biotic processes in soil. Future work should consider in more detail the composition or fractions of soil organic matter and substrates added to soil, as well as the reactions of soil microbial communities in response to biochar amendment.

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6. Degradability of raw and post-processed chars in a two-year field experiment

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Abstract

The decomposition dynamic of chars as well as possible priming effects of added char on soil C degradation were studied in a two-year experiment at an agricultural field. During the first two years after application of four chars and one digestate from maize silage (7.7 t C ha^{-1}), soil carbon respiration fluxes and the corresponding carbon isotopic abundances were determined. Applying isotopic mixing models, it was possible to distinguish and to quantify the fractions of CO_2 originating from the added substrate and the soil.

In contrast to digestate, which decayed at a constant intensity with a half-life of 14 years, the carbonised products were significantly stabilised. Pyrolysis char mineralised with a decreasing intensity over time, with an estimated half-life of 81 years. HTC char showed a high emission of CO_2 during the first year but the remaining recalcitrant pool was then

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mineralised with a half-life of 60 years. When the chars were fermented before being applied to the soil, the initial CO_2 emission from HTC was reduced but on the long term the chars were less stable and continued decaying like digestate. The effects of the added substrates on the degradation of soil organic carbon were negligible. In conclusion, both untreated types of char were sufficiently stable to justify agronomic use in order to sequester carbon in soil.

Keywords

CO_2 emission, decay dynamics, HTC char, pyrolysis char, soil carbon turnover, stable isotopes

6.1 Introduction

The application of pyrolysis char, generally defined as biochar, to soils has been proposed as a method for the long-term storage of photosynthetically fixed carbon in the environment. At the same time carbonised organic matter may provide agronomic benefits due to the improvement of soil properties (Lehmann et al., 2006; Schulz and Glaser, 2012). Biochar in soil can increase the water holding capacity, the stability of soil aggregates and the availability of nutrients, which in turn all have positive effects on plant growth and biomass yields (Biederman and Harpole, 2013; Jeffery et al., 2011; Lehmann et al., 2006).

Besides pyrolysis char, another remarkable carbonisation product is char originating from hydrothermal carbonisation (Libra et al., 2011; Titirici et al., 2012) referred to as HTC char. Compared to traditional ("dry") pyrolysis, HTC has the advantage of allowing

carbonisation of feedstocks with high water content, such as grass, litter and sewage sludge. This technique thus provides a way to sterilise and reuse these substrates for various purposes at the same time, i.e. carbon sequestration and soil amelioration. Yet HTC char has been reported to contain a considerable amount of toxic compounds, among which are phenols, toluene, furfural and furan (Bargmann et al., 2013; Becker et al., 2013; Busch et al., 2012). These compounds can have detrimental effects for plant yield, mostly in the first years after application. To improve biocompatibility of HTC chars before application, post-processing such as washing (Dicke et al., 2015b) is required. A relatively new possibility of post-processing is fermentation of chars in an anaerobic biogas reactor (Andert and Mumme, 2015; Mumme et al., 2014), which has already proved to be effective in reducing CO_2 emissions in soil-char mixtures (Lanza et al., 2015).

Besides the desirable effects onto soil quality and crop yield, chars have some less apparent effects on the abundance and composition of soil fauna and microflora (Atkinson et al., 2010; Lehmann et al., 2011). As a consequence, the presence of char might also change the degradability and turn-over of the present soil organic matter, a phenomenon referred to as “priming” (Kuzyakov, 2010). Both positive (Hamer et al., 2004; Jones et al., 2011) and negative (Whitman et al., 2014) priming effects of chars on the decay of soil organic matter have been reported (Kuzyakov, 2010; Woolf and Lehmann, 2012); in some experiments, biochar-induced priming even switched between being positive and negative during the course of time (Maestrini et al., 2014). A recent review (Wang et al., 2016) reports generally negative priming effects, especially for chars produced from crop residues on the majority of soil types, with the remarkable

exception that positive priming prevails in sandy soils. The extent and the duration of the impacts of chars on soils are strongly dependent on their degradability, the knowledge of which is therefore essential for the evaluation of the suitability of chars for carbon storage and soil amendment.

The degradation process is usually investigated indirectly, by tracing one or several fluxes from a limited matter pool over time (Becker et al., 2014; Cheng et al., 2008; Ronsse et al., 2011; Zimmerman, 2010). For the decay of a carbon-rich substrate such as a char, the mineralisation to CO_2 is the prevalent mechanism and therefore gas exchange measurements can be applied for the characterisation of the degradation dynamics of chars added to soil. Due to the possible priming effects, particular care must be taken to separate char and soil-derived CO_2, the latter including both respiration by roots and microorganisms, degrading soil organic matter. Such a separation can be achieved by the application of specific mixing models (Dawson et al., 2002; Fry, 2003), which allow to calculate the fraction coming from either source on the basis of the relative abundance of stable isotopes ($^{13}C/^{12}C$) or of radio isotopes ($^{14}C/^{12}C$) in CO_2, provided the isotopic composition of the char and soil carbon is known and sufficiently different (Phillips and Gregg, 2001). The measurement of stable isotopes, both in the gas phase and in the solid phase, has already been used successfully for a specific analysis of char-derived carbon decomposition (Bai et al., 2013; Glaser and Knorr, 2008; Jiang et al., 2016; Pan et al., 2016).

For the investigation of char degradability and effects of chars onto the soil environment, incubation of soil-char mixtures at the laboratory scale under controlled conditions is the easiest approach, which allows a mathematical description of the decay

kinetics and consequently the estimate of a mean residence time τ=*MRT* or equivalently a half-life $t_{½}$ (years). On the other hand, such approaches often do not account for the multiple interactions in the soil-plant-atmosphere continuum. Consequently, an investigation at the field scale will closer mirror reality, even though such studies are complicated by the influence and interaction of several external factors. Amongst them are variable climatic and physico-chemical conditions, the variability in amount, quality and availability of carbon, the availability of nutrients and energy sources, as well as the influence of mycorrhiza and soil fauna (Kolb et al., 2009; Li et al., 2011; Warnock et al., 2007). For the practical application of chars at a field scale, fertilisation and other common management practices play also an important role (Sun et al., 2016) and their simulation can often not be achieved adequately under laboratory conditions.

Most studies about char stability in a field environment try to deduce the degradability from char-induced variations in the soil respiration (Liang et al., 2008; Qayyum et al., 2012; Zimmerman, 2010) or from measurements of the carbon content and carbon isotopic abundance in the soil (Gronwald et al., 2016). These approaches often have either limited time resolution or lack to reconstruct the accurate dynamics of char degradation. Therefore, it is highly advisable to apply isotopic measurements to the emitted CO_2. Previous studies (Malghani et al., 2015) assessed the char decay kinetics and yielded estimates for degradation rates and half-life. Our study is based on a longer measurement period and on other assumptions than Malghani et al. (2015) about the isotopic signature of the sources, which allow to apply a complex mixing model for determining the amount of char-derived CO_2 production.

The present study thus deals with the stability of pyrolysis and HTC char in soil and with their effects on the degradation of soil carbon under field conditions, with and without post-processing. We aim to answer the following questions:

(i) What are the dynamics, and the extent of char degradation under field conditions?

(ii) Does fermentation post-processing affect the degradability of chars in the field?

(iii) Does the application of chars influence the degradation of soil carbon (priming effect)?

6.2 Materials and methods

6.2.1 Chars, digestate and fermentation post-processing

All substrates were produced from maize silage in the same way as in our previous studies (Dicke et al., 2015a; Lanza et al., 2015; Reibe et al., 2015; Sänger et al., 2016). Maize, a C_4 plant, was chosen as a feedstock for its higher natural ^{13}C abundance in comparison to C_3 plants and the related soil organic matter (Glaser, 2005). Pyrolysis char (REW, Quakenbrück, Germany) was produced in a continuous reactor (600 °C, 30 min) and quenched by means of water sprinkling. HTC char (AVA CO_2, Karlsruhe, Germany) was produced in a one-pot batch reactor (210 °C, 23 bar, 8 hours) and separated by means of a chamber filter press. Solid digestate (Biowork, Phöben, Germany) was collected from a batch-wise solid-state process mesophilic biogas reactor fed with maize silage and operating at 35 °C. A fermentation post-processing was applied to a part of the pyrolysis and HTC chars to reduce negative impacts on plants

and soil microbes (Gajić and Koch, 2012; Lanza et al., 2016), as follows: the char was mixed with the digestate in a 2:1 mass ratio and fermented for 4 weeks under mesophilic conditions (35 °C) at the biogas facilities of the Leibniz Institute for Agricultural Engineering, Potsdam, Germany (Mumme et al., 2014), yielding mixtures of fermented char + digestate, referred to as "post-processed pyrolysis char" or "post-processed HTC char". After production, the chars and the digestate were stored under an external shelter inside flexible intermediate bulk containers (FIBC) (LC Packaging International BV, Nieuwerkerk a/d IJssel, The Netherlands) for up to one month until application onto the field. The characteristics of the used chars and digestate listed in Table 6.1.

Table 6.1 – Characteristics of chars and digestate (adapted from Sänger et al. (2016)). FM = fresh matter; DM = dry matter; oDM = organic dry matter; Pyro = untreated pyrolysis char; HTC = untreated hydrothermal carbonisation char; Pyro-ferm = pyrolysis char post-processed by fermentation; HTC-ferm = hydrothermal carbonisation char post-processed by fermentation.

Material	pH	$DM_{105°C}$	oDM	C_{tot}	$\delta^{13}C$	N_{tot}	H_{tot}	O
		(g kg^{-1} FM)	(g kg^{-1} DM)	(g kg^{-1} DM)	‰	(g kg^{-1} DM)	(g kg^{-1} DM)	(g kg^{-1} DM)
Pyro	9.89	929	996.9	752	-12.8	16.5	13.4	31.6
HTC	5.25	474	999.7	646	-14.9	20.9	46.0	253
Digestate	8.26	236	997.6	401	-16.3	36.6	40.4	174
Pyro-ferm	9.52	300	997.8	558	-16.6	25.8	24.2	113
HTC-ferm	7.03	328	998.8	549	-16.4	28.8	56.8	110

6.2.2 Field design and char application

The field experiment was conducted on a field located near the Institute of Agricultural and Urban Ecological Projects (IASP) research station in Berge (Kreis Havelland,

Brandenburg, Germany, 52° 63′N, 12° 80′E), which represents a typical arable site of the glacial landscape of North-eastern Germany. The soil was a loamy sand (Haplic Cambisol) with a bulk density of 1.53 g cm^{-3} and the following texture: 712 mg g^{-1} sand (Ø > 630 µm), 222 mg g^{-1} silt (2-630 µm) and 66 mg g^{-1} clay (Ø < 2 µm). The soil pH was 6.0, and the total carbon and nitrogen contents were 7.3 mg g^{-1} DM and 0.7 mg g^{-1} DM. Cultivated crops were winter wheat (*Triticum aestivum* L.) in 2012-13, winter rye (*Secale cereale* L.) in 2013-14, oil radish (*Raphanus sativus* L. var. *oleiformis*) as a catch crop over winter 2014-2015 and maize (*Zea mays*) in 2015.

The chars were applied to the field in September 2012 in a quantity ranging between 10 and 14 t ha^{-1}, corresponding to 7.7 t C ha^{-1}, and mixed by ploughing until 0.3 m soil depth. Every year in spring, mineral nitrogen in the form of calcium-ammonium nitrate fertiliser (CAN, 5 Ca $(NO_3)_2$ NH_4NO_3 • 10 H_2O, 27 % N) was applied in two steps in a total rate of 150 kg N ha^{-1}. Other soil management operations were performed during cultivation according to local practice and as described in detail by (Sänger et al., 2016).

6.2.3 Measurement of soil carbon-isotopic composition

Soil sampling was performed every six months between October 2012 and October 2014. In each plot, five soil samples were taken with a small auger (diameter 2 cm, depth 25 cm), mixed thoroughly and transported in a cool box, air-dried, ground up to a fine powder and finally measured in an elemental analyser (Flash EA 2000 HT, Thermofisher, Bremen, Germany) coupled via a ConFlo IV interface to an isotope mass spectrometer (Delta V Adantage, Thermofisher, Bremen, Germany) in order to determine their carbon-12 content (^{12}C, in mg g^{-1} DM) and carbon isotopic signature,

which was expressed as relative deviation from a standard in per mille (‰) units, using the δ notation:

$$\delta \equiv \delta^{13}\mathrm{C} = \frac{\left({}^{13}C/{}^{12}C\right)_{\mathrm{sample}}}{\left({}^{13}C/{}^{12}C\right)_{\mathrm{VPDB}}} - 1 \quad (13)$$

where VPDB (Vienna Pee Dee Belemnite) designates the universally accepted standard, having an isotopic ratio $R_{\mathrm{VPDB}} = \left({}^{13}C/{}^{12}C\right)_{\mathrm{VPDB}} = 0.0111802$ (Brüggemann et al., 2011).

The data analysis was performed with the software R, version 3.2.0 (R core team, 2015). For each sampling day and plot, the total soil-C (C, in mg g^{-1} DM) was then obtained by inverting equation (1):

$$C = {}^{12}C \cdot \left[1 + R_{\mathrm{VPDB}} \cdot (1 + \delta)\right] \quad (14)$$

The fraction f of soil-^{12}C originating from the added substrate (char or digestate) was calculated applying a two-endmember mixing model (Dawson et al., 2002; Fry, 2003), knowing the isotopic abundance of the control soil without any substrate addition (δ_{B}, in ‰) and of the added substrate (δ_{Z}, in ‰):

$$f = \frac{\delta - \delta_{\mathrm{B}}}{\delta_{\mathrm{Z}} - \delta_{\mathrm{B}}} \quad (15)$$

which allowed calculating the quantities of C originating from the substrate (C_{Z}, in mg g^{-1} DM) and from the soil (C_{B}, in mg g^{-1} DM):

$$\begin{cases} C_Z = {}^{12}C \cdot f \cdot \left[1 + R_{\mathrm{VPDB}} \cdot (1 + \delta_{\mathrm{Z}})\right] \\ C_B = {}^{12}C \cdot (1 - f) \cdot \left[1 + R_{\mathrm{VPDB}} \cdot (1 + \delta_{\mathrm{B}})\right] \end{cases} \quad (16)$$

6.2.4 Measurement of isotopic composition of soil-respired carbon dioxide

Gas sampling was conducted once a week from April 2013 until September 2014, according to an established practice (Balasus et al., 2012; Dicke et al., 2015a; Hellebrand et al., 2003) using closed cylindrical chambers with a volume V = 0.064 m^3 placed on a collar with a water sealing. Per each plot, four air samples were collected within 60 to 120 minutes by means of a plastic syringe connected to the chamber via a three-way valve and stored into Altef bags (Alltech Grom, Worms, Germany). The molar fraction of the emitted $^{12}CO_2$ (X, in ppm) and the isotopic signature ($\delta^{13}CO_2$, in ‰) were measured using a Picarro G2201-i analyzer (Picarro Inc., CA, USA).

The CO_2-^{12}C flux ($^{12}\Phi$, in t ha^{-1} yr^{-1}) was calculated with the following equation:

$$^{12}\Phi = \frac{MM}{VM_0} \cdot \frac{T_0}{T} \cdot \frac{V}{Æ} \cdot \frac{dX}{dt} \quad (17)$$

where MM = 12.0107 g mol^{-1} is the molar mass of ^{12}C, VM_0 = 22.414 l mol^{-1} is the molar volume of an ideal gas at the reference temperature T_0 = 273.15 K, $V/Æ$ = 0.315 m is the volume/area ratio of the chambers, dX/dt (in ppm/min) is the slope of a linear fit of the molar fraction of $^{12}CO_2$ against time and T (in K) the air temperature, measured at the nearby located meteorological station of the German Meteorological Service (Station Nr. 05825, Berge, Kreis Havelland, Brandenburg, Germany, 52° 63′N, 12° 80′E).

The isotopic signature of the emitted CO_2 (δ^R, in ‰) was calculated as the slope of a linear fit of the product of molar fraction and isotopic abundance against the molar fraction, a procedure known as “Miller-Tans-plot” (Kayler et al., 2010; Miller and Tans, 2003):

(18) $$\delta^{R} = \frac{d\left(X \cdot \delta^{13}CO_2\right)}{dX}$$

The total emitted CO_2-C (Φ, in mg CO_2-C m^{-2} h^{-1}) was then equal to

(19) $$\Phi = {}^{12}\Phi \cdot \left[1 + R_{VPDB} \cdot \left(1 + \delta^{R}\right)\right]$$

The fraction f^R of CO_2-^{12}C originating from the added substrate was determined through a more complex procedure, to account for the isotopic fractionation taking place during the mineralisation of carbon from the solid to the gas phase, which can be quantified by a fractionation coefficient α (assumed to be substrate-independent). The kernel of this procedure, which to our knowledge has not been described in the literature yet, is the application of a non-linear model equation to the measured respiration isotopic abundances δ^R, relating them to the known isotopic abundance of soil and of the added substrate, δ_B and δ_Z:

(20) $$\delta^{R} \sim \alpha \cdot \left[1 + \delta_B + f^{R} \cdot \left(\delta_Z - \delta_B\right)\right] - 1$$

Solving this equation for the parameters α and f^R allowed calculating the mineralisation fluxes for the substrate-C (Φ_Z, in mg CO_2-C m^{-2} h^{-1}) and for the soil-C (Φ_B, in mg CO_2-C m^{-2} h^{-1}):

(21) $$\begin{cases} \Phi_Z = {}^{12}\Phi \cdot f^{R} \cdot \left[1 + R_{VPDB} \cdot \alpha \cdot \left(1 + \delta_Z\right)\right] \\ \Phi_B = {}^{12}\Phi \cdot \left(1 - f^{R}\right) \cdot \left[1 + R_{VPDB} \cdot \alpha \cdot \left(1 + \delta_B\right)\right] \end{cases}$$

6.2.5 Estimate of the degradation rates

The average carbon mineralisation rate k was calculated within each vegetation period as the average of the emission flux, normalised for the initially added carbon C_0 = 770 g C m^{-2}:

$$k = \frac{\overline{\Phi}_Z}{C_0} \quad (22)$$

The half-life was calculated according to the standard formula $t_{½} = (\ln 2)/k$.

6.3 Results

6.3.1 Soil-derived and char-derived carbon content

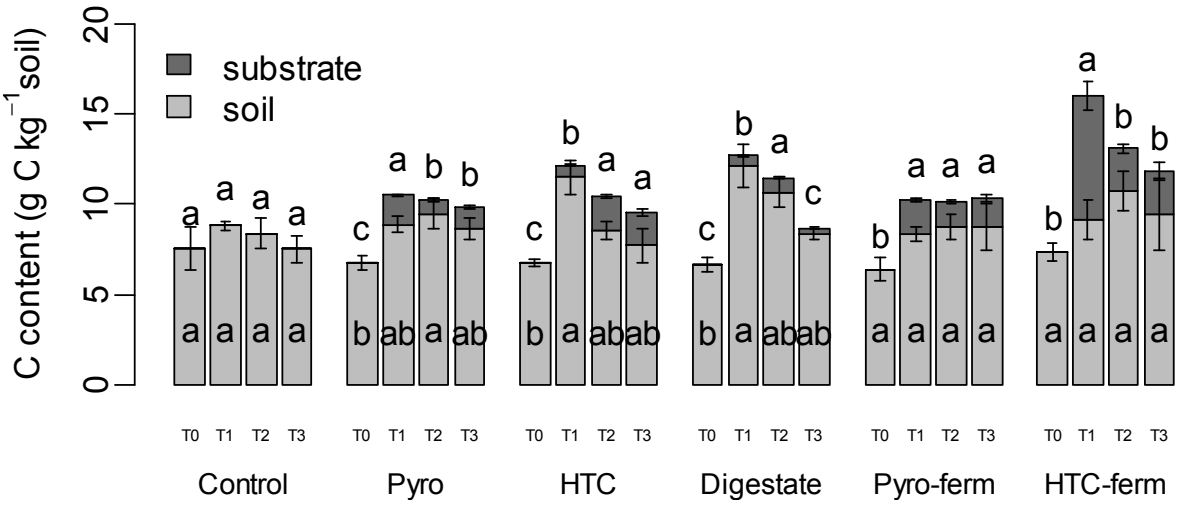

Figure 6.1 – Stocks of total soil carbon after application of chars, digestate and char-digestate mixtures at four sampling times: T0 = before char application, T1 = 22 days, T2 = 211 days, T3 = 806 days after application. The soil organic matter derived carbon is indicated in dark grey, the char derived carbon in light grey. Different letters for the same treatment indicate significant differences at P = 0.05.

Figure 6.1 shows the total carbon content of the soils with added substrates (and controls) one week before application of chars and digestate (September 2012), 20 days

after (October 2012), at the beginning of the first vegetation period (April 2013) and at the end of the second vegetation period (October 2014), separated into two components: one originating from the soil and the other from the added char or digestate.

All treatments provided an increase in soil organic matter carbon within the first month, in most cases nonsignificant, which might be due to the tillage which followed application of the substrates. During the two years of the experiment, a nonsignificant tendency of soil organic matter carbon to decrease over time of could be observed for the following treatments: control, digestate, raw and fermented HTC char. The amount in soil organic matter carbon for the treatment with raw and fermented pyrolysis char did not change at all. In general, the content of soil-derived carbon did not depend on the substrate applied, apart from the measurement 20 days after char application.

The extent of substrate-derived carbon increase depended significantly from the type of substrate applied, though in a way varying over time. Digestate was the substrate providing the least carbon amount at any time. Fermentation of chars generally did not result in any significant effect on the quantity of substrate-derived carbon.

6.3.2 Soil-derived and char-derived carbon mineralisation

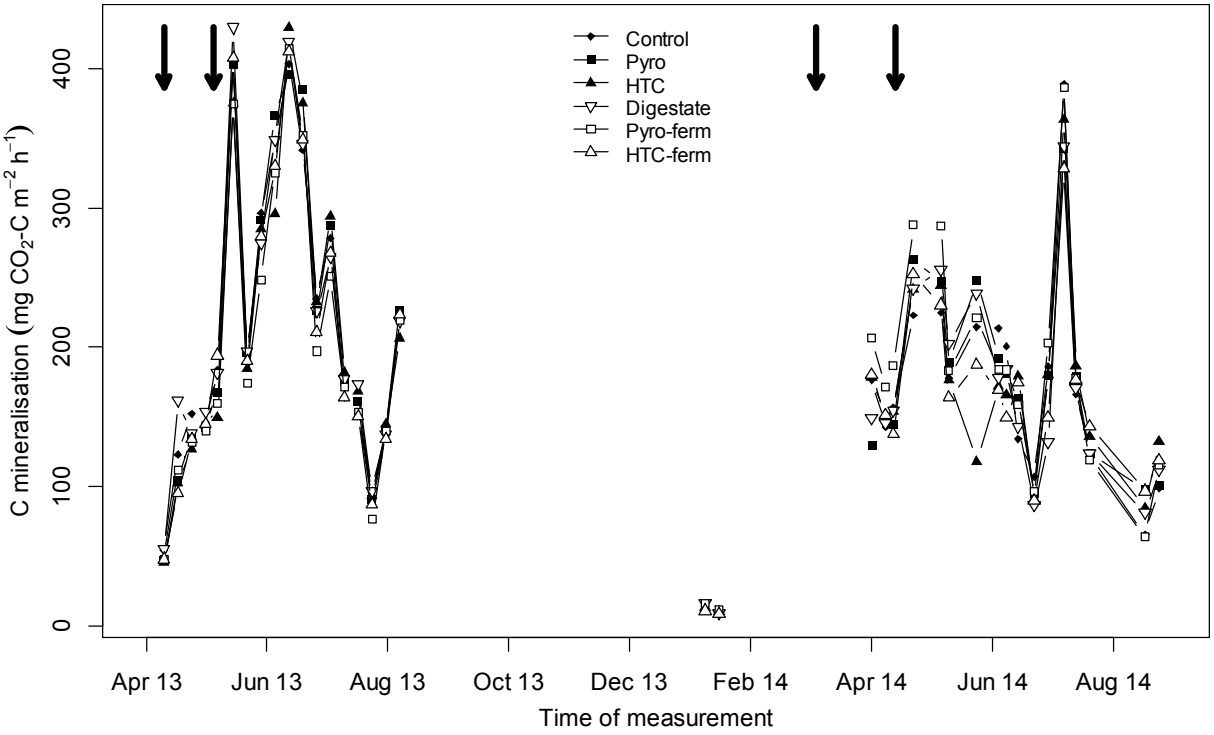

Figure 6.2 – Mineralisation dynamics of soil-derived C during two vegetation periods after application of chars and digestate. The arrows mark time points of fertilisation.

The separation of the CO_2 fluxes via the isotopic mixing model described in Section 6.2.3 was successfully applied during the two vegetation periods of this study, ranging from April to August each year, when the signal/noise ratio was high enough to distinguish the isotopic signature of the control from the other treatments. We did not perform a flux separation during the winter months, when CO_2 fluxes were low.

Figure 6.2 shows the mineralisation dynamics for soil organic matter derived C. The soil carbon mineralisation did not differ significantly among the variants with or without added substrates at any time point.

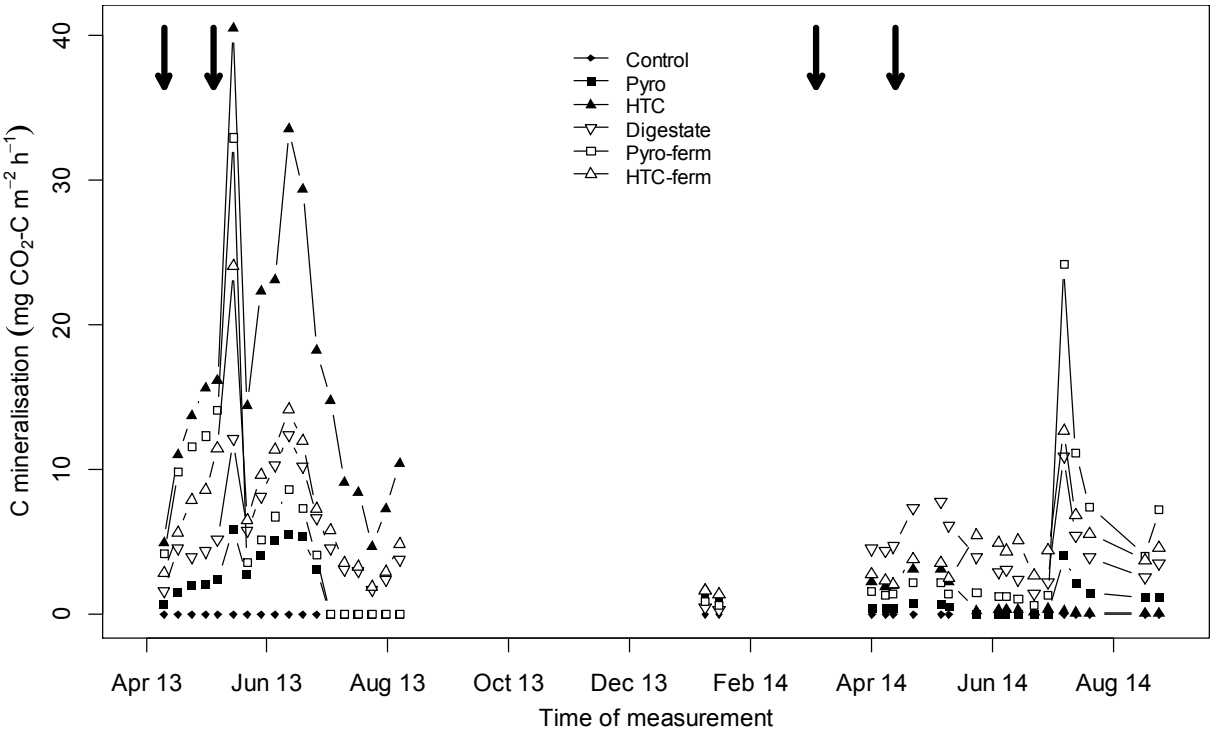

Figure 6.3 – Mineralisation dynamics for C derived from the added substrates within the first two vegetation periods. The arrows mark the time points of fertilisation.

Figure 6.3 shows the calculated mineralisation dynamics for carbon originating from the added substrates (chars and digestate), which was about one order of magnitude lower than the mineralisation fluxes of soil organic matter derived carbon. Within the first vegetation period (April-August 2013), HTC char lost the highest amount of CO_2-C and was therefore the least stable substrate, followed by fermented HTC char and digestate, fermented pyrolysis char and raw pyrolysis char. During the second vegetation period (April-August 2014), a residual mineralisation was observed in all treatments containing digestate (digestate, Pyro-ferm and HTC-ferm), whereas HTC and Pyro treatments were degraded less intensively.

Table 6.2 – Average mineralisation loss and estimated half-life for the different substrates within the first two vegetation periods. For each column, values followed by the same letter are not significantly different (at *P*=0.05).

Treatment	Average emission loss (yr^{-1})		Estimated half-life (yr)	
	1st vegetation	2nd vegetation	1st vegetation	2nd vegetation
Pyro	0.026 c	0.009 b	27	81
HTC	0.188 a	0.012 b	4	60
Digestate	0.064 b	0.052 a	11	13
Pyro-ferm	0.068 b	0.048 a	10	14
HTC-ferm	0.091 b	0.052 a	8	13

The average CO_2-C losses within the two time periods under study and the corresponding decay half-lives are reported in Table 6.2. All substrates were degraded faster during the first degradation period (119 days): the highest carbon mineralisation occurred for HTC char, with an average rate of 19 % yr^{-1}, whereas the lowest occurred for pyrolysis char, with a loss lower than 3 % yr^{-1}. Digestate and fermented chars were in between, with rates of 6 % and 10 % yr^{-1} respectively. The carbon losses during the second vegetation period (145 days) were all lower than 6 % yr^{-1}; the emission rate for pyrolysis char was reduced by a factor of three and the one for HTC char by a factor of 15, approaching the value for pyrolysis char; the rates for digestate and fermented chars diminished to a lesser degree as compared to the previous year, making them the most degradable substrates.

6.4 Discussion

6.4.1 Degradability of raw chars and digestates in soil

Degradation or mineralisation of chars is the result of different processes, among which physical processes known as "weathering" (Czimczik and Masiello, 2007), chemical oxidation (Zimmerman, 2010) or metabolic degradation by bacteria or fungi (Lanza et al., 2016; Mukherjee et al., 2016) are involved. Though the bulk of chars consists mostly of stable condensed polyaromatic structures, which are largely chemically inert over time scales of centuries, their surface possesses several functional groups which can react with water and nutrients in the soil and can also be metabolised by soil microorganisms. Microorganisms incorporate this carbon into their cells or oxidise it via respiration, releasing CO_2 into the atmosphere. Concerning the chemical composition of biochars, the labile fraction, estimated by studies of the degradation kinetics, was found to be below 6 % of the total C content (Dicke et al., 2014; Lanza et al., 2015). Chars also possess a fraction of aromatic or polyaromatic compounds (Hale et al., 2012), which are mostly degassed at the initial stage of decomposition.

The decomposition rate of pyrolysis char, tracked by means of CO_2 isotopic measurements amounted to 2.6 % yr^{-1} during the first vegetation period and was reduced thereafter. Based on this mineralisation rate, we could estimate by extrapolation a half-life of about 27 years. However, that would be a conservative estimate, because on one hand soil microbial activity is low during the cold season, and on the other hand the labile fraction of the chars is increasingly depleted with aging of the chars, leaving a recalcitrant fraction which is barely degraded. Already during the

second vegetation period the carbon loss dropped to 0.9 % yr^{-1}, so that an estimate of the lifetime at that time point would be three times as long (81 years). Thus, pyrolysis char revealed to be a highly stable form of carbon under field conditions in a typical agricultural setting in central Europe. The reported values are compatible with other publications based on studies of comparable length (Gronwald et al., 2016). In several long-term experiments (Kuzyakov et al., 2009, 2014), a progressive reduction of the emission rate over time has been recorded, which allowed the authors to restrict the incertitude of the estimates for the half-life of pyrolysis char, yielding also much higher values ($t_{½}$ = 278 years).

Mineralisation of HTC char proceeded quite differently compared to pyrolysis char in our study: the average carbon loss during the first year was seven times higher than that of pyrolysis char, but during the second year it dropped to a level comparable to pyrolysis char. This finding suggests that HTC char is a highly heterogeneous substrate containing more than 5 % of labile carbon compounds, which can be degraded very quickly within the first year, and a more recalcitrant fraction, which is mineralised in a way similar to pyrolysis char, with an estimated conservative half-life of 60 years. Other authors (Malghani et al., 2015) reported a similar biphasic tendency in the degradation of HTC char. Studies of comparable length using the same chars (Gronwald et al., 2016; Malghani et al., 2015), reported for the first 13 and 19 months after char application estimates of the half-life which are comparable with ones we found during the first year. The results presented here can also be related to the results of our laboratory incubation experiments based on soil from the same field experiment (Lanza et al., 2015), where we also found higher mineralisation rates for HTC char compared to pyrolysis char and

moreover a two-step decay kinetics for HTC char. This kinetic gives evidence for an acclimation of the microbial community over time, with a relative increase of the contribution of fungi, as previously reported (Steinbeiss et al., 2009; Titirici et al., 2012). However, the C losses from the short-term study were systematically higher compared to the field investigation, confirming the preeminent impact of the labile fraction in the initial stages of decay for both substrates (Bach et al., 2016). For the same reason, particular care must be taken when extrapolating the results of a short-term investigation to forecast long-term stability of a substrate, as some later longer-term effects could be concealed.

We also compared the decomposition of both chars with the degradation of digestate, which occurred at a roughly constant intensity, yielding an estimated half-life of about 13 years. Mineralisation of pyrolysis char was systematically slower, while mineralisation of HTC char was faster than digestate during the first vegetation period and slower during the second vegetation period, reflecting its greater heterogeneity (Falco et al., 2011; Funke et al., 2013; Titirici et al., 2008). Freshly formed HTC chars may undergo physical processing by degassing of volatile compounds, as well as microbial processing due to an enhanced amount of easily degradable compounds becoming available as substrates for microorganisms. No data were available for the first months directly after application of the substrates onto the field, when probably most of the labile compounds were respired to CO_2, and could therefore not be considered in this study. For the digestate, the comparatively low fraction of substrate-C remaining in the soil already 20 days after application, however, suggests that a considerable part was lost directly after application to the field. This assumption is supported by a study of

(Severin et al., 2016) who found the highest CO_2 emission rates within 48 hours after digestate was applied to the field. A further loss of carbon could be expected due to leaching of dissolved organic compounds, which for HTC char has been estimated as high as 10 % of the respired C (Malghani et al., 2015).

6.4.2 Effect of fermentation on the stability of chars in soil

Fermented chars are different from raw chars with respect to composition, structure and accessibility; in particular they differ in their O:C ratio, which has been shown to be a good proxy and predictor for stability (Spokas, 2010). In our study, fermented pyrolysis char was initially mineralised approximately twice as fast as the corresponding untreated char and continued to degrade at a comparable intensity during the second year, similar to fermented digestate. This finding raises the question, how degradation of fermented pyrolysis char differs from the degradation of an unfermented 2:1 mixture of pyrolysis char and digestate. Since the O:C ratio was not affected by fermentation (Table 6.1; see also Lanza et al. (2015)), one could expect that the processed char was as stable as the unprocessed mixture. Instead, the carbon loss from fermented pyrolysis char (6.8 % yr^{-1} and 4.8 % yr^{-1}) was definitely higher than a weighted mean of the carbon losses from raw pyrolysis char and digestate (3.9 % yr^{-1} and 2.3 % yr^{-1}). Thus it is obvious that fermentation decreased the stability of pyrolysis char as a whole. We can only speculate about the reason, but we might assume that fermentation results in a modification of biochar surfaces enhancing the number of functional groups, which serve as locations supporting microbial attack.

Fermented HTC char initially degraded half as fast compared to raw HTC char. Subsequently, the decomposition slowed down to an intensity similar to that of digestate, but definitely higher than for untreated char. A comparison of fermented HTC char with an untreated HTC char-digestate mixture resulted in the same ordering. The lower CO_2 release in the first year can be explained by the higher degree of labile compounds contained in raw HTC char, which are decomposed during the fermentation post-processing, as confirmed by the much lower O:C ratio of the processed char (Lanza et al., 2015). On the long term, the higher residual degradation rate of fermented HTC char compared to raw HTC char might be due to the different chemical composition of the remaining recalcitrant fraction, which possibly still contains a high fraction of digestate compounds.

6.4.3 Effect of chars on soil carbon mineralisation

Within the present study, the carbon content of original soil as well as soil carbon mineralisation were not significantly changed after application of chars at any time during the entire experiment. One reason could be the relative low application rate of chars, which was considerably lower compared to other studies (Gronwald et al., 2016; Malghani et al., 2015) and might thus be the reason for neither seeing positive (Budai et al., 2016; Cross and Sohi, 2011; McClean et al., 2016) nor negative (Lu et al., 2014; Whitman et al., 2014) priming effects. However, according to a previous work based on laboratory experiments (Lanza et al., 2016), chars from maize silage can exert a negative priming with respect to readily available carbon sources such as glucose, leading to a reduced decomposition of these compounds in the presence of the chars. In contrast, the same HTC char can also exert a positive priming onto soil carbon at high soil water

contents, especially in the presence of mineral nitrogen (Andert and Mumme, 2015). Due to the low water and carbon contents of the soil in our field experiment, the possible impacts of char addition on the microbial activity might become negligible in comparison to the impact of environmental factors, which vary extremely under field conditions. We can thus conclude that char application did not affect soil carbon turnover in the carbon-poor environment of a loamy arable soil under prevailing temperate climate conditions.

6.5 Conclusions

During the first two years after application of different chars and digestate to an arable field, untreated pyrolysis and HTC chars were degraded slowly, with conservatively estimated half-lives over 60 years. Decomposition of HTC char was significantly higher during the first vegetation period, most likely due to labile compounds, which then dominated the degradation process. The fermentation post-processing was effective in removing labile compounds present in the HTC char; however, the resulting fermented chars were much less stable than the untreated ones, and behaved similar to digestate (half-life lower than 15 years). The application of chars did not affect the dynamics of soil carbon degradation at any time and thus did not act as priming agent. The presented results show that chars do not provide any significant contribution to the CO_2 emission in the arable soil under study and can contribute as long-term carbon sinks. Whether their durability is also a guarantee for long-term effects on nutrient cycling and plant growth, needs to be a central object of future research.

Acknowledgements

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7. Synthesis

Within the present work, the degradability of selected chars and their effects on the soil microbial community have been studied both under controlled conditions at the laboratory scale and under variable conditions at the field scale. Besides the differences which obviously arose due to the different systems, the results are in agreement with the current knowledge and fit well into a coherent picture of the interaction between chars and soil ecosystems. At the same time, the adopted approach, combining soil respiration measurements, isotopic measurements and DNA fingerprinting has highlighted some less known phenomena, such as the complex dynamics of degradation of HTC char and the inhibition effect of both chars on glucose-induced changes in the microbial community. The reported results highlight the relevance of short-term interactions between chars and soil microbiota, as well the evolution of the long-term degradation of carbon and nutrients in soil.

7.1 Factors driving the degradability of chars

The stability of chars is a complex subject whose estimation is affected by several factors. Some of them are related to the environmental conditions, such as temperature, humidity, pH or the abundance of nutrients and additional energy sources. Some others are related to the experimental design, such as the scale (laboratory, pot or field), the time frame (days, years, decades) and the accessibility for plants and animals.

Chars are more stable than their feedstocks. That was shown indirectly within the short-term incubation studies, where the O:C ratio for all charred products was much lower

than for uncharred straw and the soil respiration was correspondingly reduced (Sections 4.3.1, 5.3.1; Figure 4.1).

The degradation of pyrolysis char was hardly detectable in our experiments. Within the incubation studies, CO_2 release from soil amended with pyrolysis char did not differ from the control soil (Sections 4.3.1, 5.3.1); in the field experiment, the additional information about isotopic composition in the emitted CO_2 confirmed an average carbon loss of 2.6 % yr^{-1} during the first vegetation period and of 0.9 % yr^{-1} during the second vegetation period, corresponding to an estimated the half-life of 81 years (Section 6.3.2). These values are in good agreement with previous literature (Gronwald et al., 2016; Malghani et al., 2015), although longer investigations have yielded longer estimates for the half-life of pyrolysis char, up to 280 years (Kuzyakov et al., 2009, 2014).

In contrast, HTC char induced a detectable increase in the CO_2 release in the laboratory (Sections 4.3.1, 5.3.1), yet lower than uncharred straw, and was degraded remarkably faster than pyrolysis char on the field, which is compatible with results from other studies (Bai et al., 2013; Qayyum et al., 2012). The degradation rates in the field experiment dropped from 18.8 % yr^{-1} during the first vegetation period to 1.2 % yr^{-1} during the second vegetation period, corresponding to a half-life of 60 years (Section 6.4.1). This is remarkably longer than previous results reported in the literature (Gronwald et al., 2016; Malghani et al., 2015) which were limited to shorter study periods.

The ageing of the chars decreased their stability. In fact, in the field experiment, for all substrates the average degradation rates during the second vegetation period were smaller than the ones in the first vegetation period (Section 6.3.2). The dynamics of

carbon metabolism observed in the incubation studies in the laboratory were even faster (Section 4.3.4), although a direct comparison with the emission rates measured in the field was not feasible, because the laboratory studies were not designed to separate the decay of chars from the mineralisation of soil organic carbon. The decrease of the decay rates over time is a consequence of the heterogeneous nature of chars, which contain several different carbon compounds. As time goes by, the decay rates decreased because the labile fractions were progressively lost, leaving behind the more recalcitrant fractions, which decay on longer time scales. For that reason, the decay rates measured during the second year can be considered more representative of the long-term dynamics and, therefore, allow obtaining more reliable estimates of the half-lives. The labile carbon pool of HTC char contains a particularly large amount of volatile compounds (Bargmann et al., 2013; Becker et al., 2013), which are mineralised during the first months after production of the char, giving rise to the high CO_2 emissions detected during the first year of the field experiment.

Anaerobic fermentation, which had been tried as a means to improve the characteristics of chars, removed the labile compounds present in the HTC char but decreased the long-term stability of both chars. In fact, during the first short-term experiment, the induced respiration for fermented HTC char was remarkably lower than for raw HTC char (Section 4.3.2). Consistently with that, during the field experiment, the degradation of fermented HTC char within the first vegetation period was significantly reduced compared to raw HTC char (Section 6.3.2). Instead, during the second vegetation period, a residual degradation was observed for both fermented pyrolysis char and fermented HTC char (Section 6.3.2), hinting at a contribution of the digestate, which was still

present in the fermented chars (Section 6.4.2). For fermented pyrolysis char and fermented HTC char, the half-life could be estimated to be 14 and 13 years, respectively, nearly equal to the one for digestate (13 years) but remarkably lower than the values previously shown for raw pyrolysis and HTC chars.

Due to the heterogeneity of chars and to the possibly varying environmental conditions, the decay of added carbon does not always follow a simple mathematical law like a decreasing exponential function. Hence attempts to quantify stability in terms of a half-life or mean residence time can often only yield biased estimates. However, during an incubation study under controlled conditions, some phases can be identified within the decay dynamics, for which a mono- or bi-exponential fit is applicable. That way the decay half-life of specific labile carbon fractions may be calculated. On a longer-scale experiment the situation is further complicated by seasonal variations of the microbial activity, which is higher during spring and summer. Therefore, a measurement performed over the vegetation period tends to overestimate char degradation and allows calculating a lower bound for the half-life which can be considered satisfactory for practical purposes. Though permanent stability is more strictly related to long-term dynamics, early metabolic effects are important to determine the adaptation of the ecosystem to the newly added substrate and its subsequent decay, as shown by the short-term approach. Therefore, each experimental method is effective for comparing stability and biological effects of different substrates within the timescale of the experiments, but care must be applied while trying to extrapolate the results to the actual long-term stability.

7.2 Effects of chars on soil ecosystems

Biotic effects have a key role in the degradation of soil organic matter and possible additives. Within this frame, microbes are the main actors, as they possess a differentiated enzymatic apparatus, which allows them to metabolise exotic carbon compounds like the ones contained in the chars; moreover, they can react very quickly to any external matter or energy inputs. Therefore, any modification of the microbial community induced by the addition of a new substrate has consequences on the durability of the substrate itself but also on the intensity of the expected effects onto the cultivation crop.

In general, raw chars have the potential to reduce the availability of nutrients for soil microbes. This became clear during the first incubation study, when, contrary to all expectations, the added mineral nitrogen did not increase soil respiration (Section 4.4.2). It can be assumed that the added nitrogen was immobilised by the chars (Clough et al., 2013), due to their high cation exchange capacity. That would confirm also the claim that char materials can "store" nutrients and release them slowly over time (UBA, 2016, p. 83).

Chars can also "stabilise" the soil ecosystem by counteracting the variations in the microbial population structure induced by new energy input. That became apparent in the second incubation study, which compared a carbon-poor and a carbon-rich environment: the presence of chars strongly reduced the glucose-induced increase in the amount of Actinobacteria and the consequent decrease of fungi (Section 5.4.2), coherently with previous results (Steinbeiss et al., 2009; Titirici et al., 2012).

Chars can also affect the metabolism of soil organic carbon, depending on its abundance and quality. In a carbon-rich environment, chars can exert negative priming on the degradation of labile forms of carbon, as happened in the short-term laboratory incubation with added glucose (Section 5.4.2). On the other hand, in a carbon-poor environment, as it is typical in the sandy soil of Brandenburg, the net priming effect is zero or negligible, as shown by the two years of measurement in the field experiment (Section 6.4.3).

In general, HTC char had higher impacts than pyrolysis char on the microbial community, carbon metabolism and nutrient availability, which is probably due to its higher content of labile carbon compounds or its low pH value, which facilitates its metabolisation by fungi. An adaptation of the microbial community induced by HTC char was revealed also by the peculiar respiration dynamics in the short term, which showed a net increase in the respiration rate at a "breakpoint" occurring 4 to 6 days after char application.

7.3 Validity of the experimental approaches

Short-term incubations have been used to investigate the early dynamics of carbon degradation in soil-char mixtures, as well the response of the microbial community to the new substrate. The high time resolution of the respiration measurements has helped discovering fine details in the decay kinetics; the DNA amplification via real-time quantitative polymerase chain reaction has allowed highlighting the correlations between carbon metabolism and microbial community.

The field experiment was set up to investigate the degradation of chars and their environmental effects over a longer timescale and under conditions corresponding to common agricultural practice. The chosen experimental method of gas isotopic measurements allowed a separation between mineralisation of char-originating carbon and soil-originating carbon, via application of a new mixing model which extends common two-end member mixing models by accounting for metabolic fractionation during oxidation of solid carbon to CO_2 (Section 6.2.4). The extent and time resolution of the measurements are much higher than those reported so far in the literature for comparable studies with the same technique (Malghani et al., 2015; Ventura et al., 2015). The determination of the experimental procedure (collection, storage and measurement of the samples) and the derivation of the mixing model are also a major outcome of this thesis. In particular, the modified mixing model presented here (Section 6.2.4) introduced a new method to reconstruct the isotopic abundances of the two components (soil-originating and char-originating CO_2) knowing the isotopic abundances of the corresponding sources in solid form (soil and char). The key was the introduction of a substrate-independent fractionation coefficient as an additional parameter in the mixing model equation (Equation 20), which was then fit to the data to achieve the separation of the CO_2 derived from the mineralisation of chars from the CO_2 derived from the mineralisation of soil organic carbon.

The approach adopted for the present work, combining short-term incubations and a field experiment, has yielded important results on the stability of chars in soil and about their interactions with the soil ecosystem. The different experimental methods have consented to highlight phenomena occurring on different timescales and to pinpoint

some peculiarities which deserve attention and might become the object of future research. For instance, the heterogeneous nature of HTC chars stimulates further investigations to identify the single carbon components and their degradation dynamics, complemented by a study of the evolution of the soil microbial community at a higher time resolution and taxonomic specificity. On the other hand, the variability of the CO_2 emission rates in the field investigation requires a deeper understanding of the effects of soil and climatic parameters, which might become the object of a new investigation.

The short-term incubation approach, possibly in combination with gas isotopic measurements, can therefore become a benchmark to test the degradability and the effects on the soil microbial community of new types of chars, also in combination with different additives. This way a screening of new substrates can be performed in a comparatively short time, by comparing them with other already tested, which will be assumed as a reference. The effective durability and environmental effects of the chars which have been selected must subsequently be tested through a field investigation, which is the necessary complement of this procedure, as it consents a test on a longer time scale and under common practice conditions.

7.4 Conclusions

The interactions of chars with soil ecosystems are considerable and must, therefore, be carefully considered for an evaluation of the suitability of chars for the carbon storage and soil amendment. Within the present work these effects were investigated in both directions: (1) Which factors have an influence on the degradation/stability of the

chars? (2) What are the effects of the char addition on the soil microbial community and soil carbon degradation?

Both chars have demonstrated improved stability and reduced effects on the soil microbial community compared to their feedstock. The effective length and intensity of the interactions between chars and soil ecosystem depend on several parameters related to the char quality, soil quality and climate. Char stability is affected by the production process (pyrolysis char is more stable than HTC char), by the ageing process (the substrate becomes more stable over time), by the fermentation post-processing (fermented chars are less stable, though a reduced amount of volatile compounds in fermented HTC char). The effects of chars on the soil microbial community include an increase of soil respiration for HTC char over the short period, a reduction of the microbial activity and of the population shifts induced by labile carbon and a reduction of nutrient availability.

In conclusion, carbonisation of biomass residues can be an effective way to convert them into a product that is capable to store carbon and soil nutrients on a longer timescale. However, a deeper understanding of the induced microbial evolution, especially for HTC char, is required in view of their application as a soil amendment.

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Zusammenfassung

Untersuchungsgegenstand der vorliegenden Dissertation sind biomassebasierte Kohlen (Biokohlen, *biochar*), welche für eine langfristige Kohlenstoffspeicherung in Böden mit dem gleichzeitigen Ziel der zusätzlichen Bodenverbesserung hergestellt werden. Die Auswahl der Kohlen umfasste Kohlen aus Pyrolyse- und hydrothermale Carbonisierung (HTC). In dieser Arbeit werden einige zentrale Phänomene, die bei deren Ausbringung in einem bestehenden Bodenökosystem auftreten können, nähergehend untersucht. Einerseits beeinflusst das fremde Material den Stoffwechsel und die Abundanz und Vielfalt innerhalb der mirkobiellen Gemeinschaft im Boden; im Gegenzug spielen die Mikroorganismen eine aktive Rolle beim Abbau des neuen Substrats. Diese beiden Aspekte sind größer Bedeutung, um bewerten zu können, wie erfolgsversprechend der Einsatz einer bestimmten Kohle im Boden hinsichtlich der Langlebigkeit, der gewünschten Ertragseffekte sowie möglicher Nebenwirkungen ist. Daraus ergeben sich die beiden folgenden zwei Fragestellungen, auf die diese Arbeit fokusiert ist:

- Welche Faktoren beeinflussen die Abbaubarkeit der Kohlen im Boden?
- Welche Wirkungen haben die Kohlen auf die Bodenatmung, auf den Boden-C-Gehalt, auf die mikrobielle Abundanz und auf die Dynamik der mikrobiellen Gemeinschaft?

Als mögliche Einflussgrößen für die Abbaubarkeit der Kohlen wurden die Art der Kohlenherstellung, eine mögliche Nachbehandlung, der Alterungsprozess sowie die Zugabe einer Nährstoff- und einer labilen Kohlenstoffquelle getestet.

Für diese Studie wurden Pyrolyse- und HTC-Kohlen aus Mais-Silage in einen Sandboden ausgebracht. Grundlage aller Versuche war die Untersuchung der Respirationsdynamik in unterschiedlichen Boden-Kohle-Gemischen, die durch Infrarotspektrometrie ermittelt wurde. Sie diente als Indikator für die mikrobielle Aktivität und dem daraus resultierenden Abbau der Substrate. Ergänzend wurde am Anfang und am Ende jedes Versuchs der Boden-Kohlenstoffgehalt gemessen. Die Versuche erfolgten auf verschiedenen Skalen:

- Kurzzeit-Laborinkubationen (10 Tage) unter konstanten klimatischen Bedingungen in einem automatisch gesteuerten Durchflusssystem, an das das Messgerät direkt angeschlossen wurde.

- Parzellenversuch (2 Jahre) im Freiland im Nordwesten Brandenburgs, bei dem die Bestimmung der Bodenatmung mittels wiederholter Beprobung aus auf der Ackerfläche gestellten geschlossenen Hauben erfolgte.

In einer Laborinkubation wurde zusätzlich eine qPCR (quantitative Echtzeit Polymerase Kettenreaktion) zur Bestimmung der Abundanz ausgewählter mikrobieller Gruppen eingesetzt. Im Feldversuch wurde außerdem die Abundanz der stabilen Kohlenstoff-Isotopen (^{12}C und ^{13}C) im Boden und im freigesetzten CO_2 ermittelt, um den Abbau der Kohlen vom Abbau des bodenorganischen Kohlenstoffs, der durch die Kohlen beeinflusst sein kann (*priming*), zu unterscheiden.

Die Ergebnisse bestätigen die erhöhte Stabilität beider Kohlen im Vergleich zum Ausgangsmaterial, vor allem für die Pyrolyse-Kohle, deren Abbau sowohl im Labor als auch im Freiland am langsamsten erfolgte. Bei beiden Kohlen sank die Abbaubarkeit mit

ihrer Alterung. Anhand der Abbauraten im zweiten Jahr des Feldversuchs wurden für die Pyrolyse- und HTC-Kohle Halbwertszeiten von 81 bzw. 60 Jahren ermittelt. Im Gegensatz zur Pyrolyse-Kohle wies der Abbau der HTC-Kohle eine komplexere Dynamik auf, was im Lauf der 10-tägigen Inkubationsversuche mit einer Verschiebung der mikrobiellen Gemeinschaft einherging. Im ersten Jahr des Freilandversuchs kam es bei der HTC-Kohle zur Ausgasung flüchtiger und leicht abbaubarer Kohlenstoffverbindungen, wodurch die Stabilität im Folgejahr deutlich erhöht wurde.

Eine Nachbehandlung der Kohlen durch anaerobe Fermentierung führte zu einer deutlichen Verminderung der kurzzeitigen Ausgasung bei HTC-Kohle, sowohl im Freiland als auch im Labor, jedoch zu einer langfristigen Reduktion der Stabilität beider Kohlen: die ermittelten Halbwertszeiten für die fermentierte Pyrolyse- und HTC-Kohle nach dem zweiten Jahr des Feldversuchs betrugen 14 bzw. 13 Jahren.

Die Wirkung der unbehandelten Kohlen auf die Abundanz der untersuchten mikrobiellen Gruppen im C-armen Boden war stark reduziert im Vergleich zum Ausgangsmaterial, und unter C-reichen Bedingungen kam es zu einer Hemmung der Aktivitätssteigerung. Die Zugabe leicht verfügbaren Kohlenstoffs wie Glukose zum reinen Boden in einem Inkubationsversuch steigerte die Bodenatmung erheblich und erhöhte die Variationsbreite der mikrobiellen Gemeinschaft. In Gegenwart der Kohlen war dies allerdings weniger stark ausgeprägt. Bei Zugabe mineralischen Stickstoffs in Gegenwart von Kohlen wurde hingegen keine signifikante Veränderung der Bodenatmung nachgewiesen.

Die Inkubationsversuche haben es ermöglicht, die Kurzzeitdynamik der Bodenatmung und die Anpassung der mikrobiellen Gemeinschaft nach Zugabe der Kohlen und

zusätzlicher C- und N-Quellen nachzuweisen. Im Freilandversuch konnte die Abbaudynamik von Kohlenstoffverbindungen unter Praxisbedingungen untersucht werden und durch die Messung der stabilen Isotope differenzierte Aussagen über die langfristige Stabilität von zugesetzten Kohlen und der bodenorganischen Substanz getroffen werden.

Eine langfristige Festlegung von Kohlenstoff ist im Boden in Form von Biokohlen ist möglich. Allerdings hängt die Dauer der Festlegung von einer Vielzahl von Faktoren wie der Art der Ausgangsstoffe, den Prozessbedingungen, den Interaktionen zwischen Kohlepartikeln und Bodenorganismen und nicht zuletzt der Versuchsdauer ab. Während Kurzzeitversuche eine gute Möglichkeit darstellen, um die Effekte veränderter Bedingungen im Boden aufzuzeigen, kann die Kohlestabilität im Boden und damit das C-Sequestrierungspotenzial am zuverlässigsten nur in Langzeitstudien im Freiland abgeschätzt werden.

Riassunto

Oggetto della presente tesi sono i carboni prodotti da biomasse (*biochar*) e utilizzati per lo stoccaggio del carbonio nel suolo e allo stesso tempo come ammendanti per terreni agricoli. I carboni considerati sono derivati da pirolisi o carbonizzazione idrotermale (HTC). Nel presente lavoro vengono esaminati in dettaglio alcuni importanti fenomeni che si possono manifestare in seguito all'applicazione su un ecosistema preesistente nel terreno: da un lato il materiale estraneo influenza il metabolismo della materia organica, l'abbondanza e la varietà all'interno della comunità microbica nel suolo, dall'altro i microorganismi giocano un ruolo fondamentale nella degradazione del nuovo substrato. Questi due aspetti sono essenziali per valutare quanto sia opportuno l'utilizzo di un determinato carbone dal punto di vista della sua longevità, degli effetti attesi sulla resa agricola e di eventuali effetti collaterali sull'ecosistema. Da queste premesse sono emerse le seguenti domande, su cui è focalizzato il presente lavoro:

- Quali fattori determinano la degradabilità dei carboni nel suolo?
- Che effetti possono avere i carboni sulla respirazione del terreno, sul suo contenuto di carbonio, sull'abbondanza dei microorganismi e sulla dinamica della comunità microbica?

Come possibili variabili indipendenti per la degradabilità dei carboni sono state considerate: il processo di produzione, un possibile posttrattamento, l'invecchiamento dei substrati, l'aggiunta di nutrienti e di carbonio biodisponibile.

Per questo studio sono stati applicati carboni derivati da pirolisi e da HTC di insilato di mais in un terreno sabbioso. Il fondamento di tutti gli esperimenti riportati è lo studio della dinamica della respirazione microbica in diverse miscele terreno/carbone, misurata tramite spettroscopia a infrarossi, che vale come tracciante per l'attività microbica e per la degradazione del substrato. In aggiunta è stato periodicamente misurato il contenuto di carbonio nel terreno. Gli esperimenti sono stati condotti su due diverse scale:

- Incubazioni in laboratorio (10 giorni) in condizioni climatiche controllate, all'interno di un apparato per la ventilazione a flusso continuo, con presa diretta per lo strumento di misura.

- Esperimento in campo parcellizzato (2 anni) nel Brandeburgo nordoccidentale, dove la misura della respirazione è avvenuta per campionamento ripetuto da camere opache poggianti ermeticamente sul suolo.

Per una delle incubazioni è stata anche eseguita una qPCR (reazione a catena della polimerasi quantitativa in tempo reale) per quantificare l'abbondanza di determinati gruppi tassonomici di microorganismi. Nel campo è stata inoltre misurata l'abbondanza degli isotopi stabili del carbonio (^{12}C e ^{13}C), sia nel terreno sia nella CO_2 liberata, per differenziare la degradazione dei carboni da quella del carbonio organico nel suolo, che in principio può essere influenzata dalla presenza dei carboni (*priming*).

I risultati confermano l'aumentata stabilità di entrambi i carboni in confronto al materiale di partenza, in particolare del carbone pirolitico che si è degradato piú lentamente, sia in laboratorio sia in campo. La degradabilità di entrambi i carboni si è in

ogni caso ridotta con l'invecchiamento. Basandosi sulle emissioni del secondo anno della sperimentazione in campo, sono stati calcolati dei tempi di dimezzamento di 81 anni e 60 anni, rispettivamente per il carbone pirolitico e per il carbone da HTC. La degradazione del carbone da HTC ha rivelato una dinamica piú complessa, che testimonia un adattamento della comunità microbica nell'arco dei 10 giorni di incubazione. Nel primo anno in campo è stata rilevata un'elevata emissione di composti volatili e labili, che ha portato a un incremento della stabilità nell'anno seguente.

Il posttrattamento dei carboni tramite fermentazione anaerobica ha comportato una notevole riduzione dell'iniziale mineralizzazione del carbone da HTC, ma una diminuzione della stabilità di entrambi i carboni sul lungo periodo: i tempi di dimezzamento calcolati per il carbone pirolitico fermentato e per il carbone da HTC fermentato nel secondo anno dell'esperimento sul campo valgono rispettivamente 14 anni e 13 anni.

Nel terreno usato, povero di carbonio, gli effetti dei carboni sull'abbondanza dei gruppi microbici selezionati è stata nettamente ridotta rispetto al materiale non carbonizzato, mentre la reazione all'aggiunta di carbonio labile è stata tendenzialmente inibitoria. Infatti, se l'aggiunta di glucosio ha incrementato considerevolmente la respirazione e l'ampiezza delle variazioni nella comunità microbica, in presenza dei carboni le variazioni sono state fortemente ridotte. L'aggiunta di azoto inorganico non ha invece portato a variazioni apprezzabili nella respirazione.

Gli esperimenti basati su incubazioni hanno consentito di determinare la dinamica a breve termine della respirazione e l'adattamento della comunità microbica in seguito ad aggiunta dei carboni e di altre sorgenti di carbonio e azoto. Nell'esperimento su campo si

è potuta osservare la dinamica di degradazione dei composti carboniosi in condizioni di prassi agricola e grazie alla misura degli isotopi stabili si sono potuti ottenere risultati differenziati sulla stabilità a lungo termine dei carboni e della sostanza organica del suolo.

È quindi possibile immagazzinare il carbonio in modo duraturo nel suolo sotto forma di carbone. La durata stimabile di questo immagazzinamento dipende però da molteplici fattori tra cui la materia prima, il processo di carbonizzazione, le interazioni tra particelle carboniose e microorganismi del suolo e non da ultimo la durata della sperimentazione. Gli esperimenti a breve termine sono un mezzo efficace per rilevare le conseguenze immediate di modifiche del terreno; la stabilità dei carboni e quindi il loro potenziale per il sequestro del carbonio può essere determinata nel modo piú affidabile solo in studi a lungo termine sul campo.

Summary

The object of the present thesis is charred biomass (biochar) produced for double aim of carbon storage in soil and improvement of soil properties. The chosen chars included chars from pyrolysis and hydrothermal carbonisation (HTC). The present work investigates closely some basic phenomena which can occur upon application of chars into an existing soil ecosystem: on the one hand, the allochthonous material affects the metabolism and the relative abundance of different microbial groups; on the other hand the microorganisms play an active role in the degradation of the new substrate. These two aspects are crucial to evaluate the suitability of the application of a specific char in the soil, particularly as concerns its stability, the length of time the char remains in the soil, the expected effects on crop yields, as well as possible side effects on the soil ecosystem. Based on this, two research questions arise which have been investigated in this thesis:

- What factors affect the degradability of chars in soil?
- How do the chars influence soil respiration, soil carbon content, microbial abundance and the dynamics of the microbial community?

The production process, a post-treatment, the ageing process as well as the addition of a source of nutrients and a source of labile carbon were assessed as possible factors in determining the degradability of chars.

For the present study, pyrolysis char and HTC char from maize silage were applied to a sandy soil. The basis of all experiments was an investigation of the respiration dynamics

in different soil/char mixtures, measured through an infrared spectrometer, which was used to track the microbial activity and the substrate degradation. As a complement, soil carbon was also measured at the beginning and at the end of each experiment. The investigations were performed at different scales:

- Short-term laboratory incubations (10 days) under constant climatic conditions in an automatic multi-channel flowthrough system, with direct plug-in for the measurement instrument.

- A plot-wise investigation (2 years) in an agricultural field in North-West Brandenburg, where the soil respiration was measured by a repeated sampling from static chambers placed hermetically on the field.

For one incubation study, qPCR (qunatitative real time polymerase chain reaction) was additionally applied to determine the abundance of selected microbial groups. Moreover, for the field investigation the abundance of stable carbon isotopes (^{12}C und ^{13}C) in the soil and in the released CO_2 was recorded, to differentiate between the degradation of the chars and the degradation of soil organic carbon, which might be affected by the presence of chars (priming).

The results confirm the higher stability of both chars in comparison to the feedstock, in particular for pyrolysis char, whose decay was the slowest both in the laboratory and in the field. The degradability of both chars decreased with their ageing. Based on the decay rates in the second year of the field investigation, decay half-lives for pyrolysis char and HTC char amounted respectively to 81 years and 60 years. Other than pyrolysis char, the degradation of HTC char revealed a more complex dynamics, which was

accompanied by a shift of the microbial community within the 10 days incubation. During the first year of the field experiment, an intensive release of volatile and labile compounds took place, which led to an increased stability during the following year.

A post-treatment of the chars via anaerobic fermentation led to a reduction in the initial degasing of the HTC char, both in the laboratory and in the field, but also to a decrease in stability for both chars: the calculated half-lives for fermented pyrolysis char and fermented HTC char on the basis of the second year of the field investigation were respectively 14 years and 13 years.

The effects of the untreated chars on the abundance of the selected microbial groups in the carbon-poor soil used was also strongly reduced in comparison to the feedstock, while in a situation of carbon abundance a inhibition of the activity increase took place. Addition of readily available carbon in the form of glucose increased soil respiration tremendously and magnified the variation amplitude of the microbial community, which was however much reduced in the presence of chars. Instead, after addition of mineral nitrogen in presence of chars, no significant variation in the soil respiration could be observed.

The incubation experiments made it possible to report the short-term dynamics of the soil respiration and the adaptation of the microbial community after application of char and additional carbon and nitrogen sources. In the field experiment the decay dynamics of char compounds could be investigated in a situation of common agricultural practice and the measurement of stable isotopes has given differentiated outcomes about the long-term stability of the added chars and of the soil organic matter.

Storage of carbon in the soil in the form of char for a long period is possible. How long carbon can actually be stored depends on a number of factors such as the feedstock, the carbonisation process parameters, the interactions between char particles and soil microorganisms and the duration of the investigation itself. Short-term experiments represent a good possibility to highlight the effects of modified soil conditions, while the stability of char in soil and thus the potential carbon sequestration can be estimate in the most reliable way only through long-term studies in field.